

Antioxidant and Antibacterial Activity of Basil (*Ocimum basilicum* L.) Essential Oil in Beef Burger

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ABSTRACT

The aim of this study was evaluation of phytochemical components, antioxidant activity, and antibacterial effects of basil (*Ocimum basilicum* L.) essential oil (BEO) *in vitro*. The lipid oxidation of the meat and antibacterial effects of BEO were also evaluated in beef burger product. In this empirical study, essential oil of the basil was isolated by hydrodistillation. Then, BEO was analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS). The effect of different concentrations of BEO (0.00, 0.062, 0.125, and 0.25%) at 4±1°C temperature and storage time of up to 12 days was evaluated on lipid oxidation, anti *Staphylococcus aureus* activity, and organoleptic effects in beef burger. The main compounds in BEO were methyl chavicol (85.19%), 1,8 cineol (3.96%), trans-alpha bergamotene (1.18%) and linalool (1.03%). In the storage temperature (4±1°C), the BEO decreased the growth rate of *S. aureus* in beef burger (P< 0.05). Also, overall acceptance rate in the beef burger containing 0.125% BEO created a better sense in the product (P< 0.05). No significant differences were observed after adding different concentrations of essential oil to decrease lipid oxidation in raw beef burger (P> 0.05). Therefore, this essential oil might be used as antibacterial agent and flavor enhancer in meat products such as beef burger.

Keywords: Flavor enhancer, Gas chromatography, *Ocimum basilicum*, Phytochemical components.

INTRODUCTION

Today, there is a great interest in replacing the synthetic preservatives with aromatic compounds like essential oils in foods. The food industries researchers search for superseded sources of antibacterial and chemical preservatives against inroad of bacteria and lipid oxidation in foods (Guimarães *et al.*, 2010).

Recently, the extracts and essential oils of medicinal plants have been considered not only for prevention (Baradaran *et al.*, 2012) or treatment (Asgary *et al.*, 2013) of various

diseases, but also as natural food preservatives. Most of these effects have been attributed to their antioxidant activities (Rafieian-Kopaei, 2012).

Basil (*Ocimum basilicum*) is one of these medicinal plants and is called "Reyhan" in Iran. This genus is of the *Lamiaceae* family and its dried leaves as well as its essential oil are used in food industry as aromatic and flavoring ingredients. Also, *Ocimum basilicum* is commonly used to treat fever, inflammatory, stomach ache, flatulence, constipation and is also used as an antibacterial and antifungal agent (Rafieian-

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Kopaei and Hosseini-Asl, 2005; Özcan and Chalchat, 2002).

Previous studies have demonstrated the antioxidant and antibacterial activities of essential oil on different kinds of plants as basil in vitro (Bunrathep *et al.*, 2007; Kordali *et al.*, 2005, Lee *et al.*, 2005; Sokmen *et al.*, 2004; Özcan and Chalchat, 2002).

Meat and its products as beef burger are widely consumed all over the world. During storage time, the shelf life of these products is reduced. Oxidation of the lipids causes the degradation of organoleptic properties which are present as flavor, color, and texture in meat products (Salem *et al.*, 2010). Also, food spoilage and pathogenic bacteria can contaminate meat products and lead to public health hazard and economic losses (Salem *et al.*, 2010). On the other hand, use of the chemical antioxidants with high activity, such as tertiary butyl hydroquinone (TBHQ), with carcinogenic property can threaten consumers' health (Ayoughi *et al.*, 2011).

Therefore, the aim of this study was evaluation of phytochemical components, antioxidant activity, and antibacterial effects of basil (*Ocimum basilicum*) essential oil (BEO) in vitro. The lipid oxidation of the meat and antibacterial effects of BEO were also evaluated in beef burger product in refrigerated condition.

MATERIALS AND METHODS

Preparation of the Essential Oil

Samples of Basil (*Ocimum basilicum*) were collected from Charmahal va Bakhtiari Province of Iran and identified by the standard botanic work in Medical Plants Research Center in Shahre-Kord University of Medical Sciences, Iran.

The dried leaves of the plant (100 g) were hydro-distilled for 3 hours using a Clevenger type apparatus. Then, the essential oil was dried with anhydrous sodium sulfate and kept in sealed vials at 4°C (Busatta *et al.*, 2008).

Chromatographic Analysis

Gas Chromatography (GC) Analysis

The essence produced from basil 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⁻¹ (Saatchi *et al.* 2014). The essential oil was diluted in n-pentane (1/1,000, v/v) and 1.0 µL injected in the splitless mode. The primary oven temperature was maintained at 50°C for 5 minutes and then increased to 240°C at the rate of 3 °C min⁻¹. Temperatures of injector and detector were 290 and 300°C, respectively. Quantitative data were obtained from GC peaks area percent.

Gas Chromatography/Mass Spectrometry (GC/MS)

An Agilent 6890 GC system with a HP-5MS capillary column (30 m×0.25 mm, film thickness 0.25 µm) fitted with an Agilent HP-5973 mass selective detector was used for GC/MS analysis. The electron ionization (EI) system with ionization energy of 70eV 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 amu range.

Compounds Identification

Identification of constituents of essential oil was achieved by retention indices (determined with homologous series of n-alkanes C8-C20, under similar conditions) and compared their RI with data reported in the articles, references books, as well as standard libraries (Wiley275.L and Wiley7n.L) (Adams, 1995).

Determination of Antioxidant Activity

The total antioxidant capacity of *Ocimum basilicum* essential oil was assayed using the

β -carotene and linoleic acid (Shirzad *et al.*, 2011).

Total Phenol Determination

The amount of total phenolic content in the obtained essential oil was estimated by Folin Ciocalteu method (Salmanian *et al.*, 2014).

Determination of Antimicrobial Activity

The antimicrobial activity of *Ocimum basilicum* essential oil was assayed using micro-dilution method (Sharafati-chaleshtori *et al.*, 2013).

Preparation of Beef Burger

Five kg beef burger was taken from a batch production of a meat products factory. For microbiological analysis samples (100 gr) 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 bacterial growth.

The samples were divided into treated and untreated groups (control). The treated groups were added 0.062, 0.125, and 0.25% (v/w) concentrations of essential oil of basil. All samples were labeled and stored at 4°C as well as each sample analysis days (0, 1, 3, 6, 9 and 12) for chemical and microbiological factors (Noori *et al.* 2011).

Determination of Lipid Oxidation

Thiobarbituric acid reactive substances (TBARS) assay was conducted as described by Maraschiello *et al.* (1999) with some modifications. To this end, 0.5 g of raw beef burger samples was added to 10 mL of deionized water and vigorously mixed for 1

min and then 2.5 mL of 25% TCA (Trichloroacetic acid) were added. The samples were stored for 15 min at 4 °C and centrifuged for 5 min (4,000 rpm, at 4°C). The 3.5 mL of supernatant with 1.5 mL of 0.6% TBA was mixed 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 H₂O, 1 mL 25% TCA, and 1.5 mL 0.6% TBA. The standard curve was prepared by standard solutions 1, 1, 3, 3-tetraethoxypropane (TEP) (Sigma-Aldrich Corporation, St. Louis, MO., USA). The amount of TBARS was expressed as mg of malonaldehyde (MA) per kg of meat (Maraschiello *et al.* 1999).

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 (1189 with PTCC *Staphylococcus aureus*) was moved to Brain heart infusion (BHI) and was incubated at 37°C for 18 hours. Then, from previous suspension was again cultured in BHI. The growth of *S. aureus* with concentration of 1.5×10^8 colony forming unit cfu mL⁻¹ in the broth was adjusted using the spectrophotometer at 600 nm (Unico UV-2100, USA). The number of bacteria in the BHI was also counted by surface cultivation after 18 hours incubation at 37°C. Finally, beef burgers samples were inoculated with pathogen cells of 10^3 cfu g⁻¹ of *S. aureus* (Shekarforoush *et al.*, 2007).

Sensory Evaluation

A hedonic test was used for sensory evaluation described by Amanya *et al.* (2012). The beef burger were cooked in a preheated electrical grill for a total of 4



minute and the cooked samples were served warm to 6 members of household trained panel regardless 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 the present study were framed as means±standard deviation of

triplicates. The significance of difference test was performed by Friedman, Kruskal-Wallis and Dunn's Multiple Comparisons test using INSTAT software. $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

In Table 1, the constituents of essential oil obtained by GC/MS can be observed. Twenty five compounds were identified in which the highest ones were methyl chavicol (85.19%), 1,8 cineol (3.96%), trans-alpha bergamotene (1.185%), linalool (1.03%), eugenol (0.7%), and γ -terpinene (0.53%).

Table 1. The chemical components of *Ocimum basilicum* essential oil.

No	Retention Time (RT)	Kovats Indices (KI)	Components	Concentration (%)
1	11.22	926	α -pinene	0.12
2	13.32	969	β - pinene	0.28
3	14.14	986	β -myrcene	0.08
4	15.84	1020	<i>p</i> -cymene	0.22
5	16.20	1027	1,8 cineole	3.96
6	17.09	1045	<i>z</i> -beta-ocimene	0.24
7	17.59	1055	γ -terpinene	0.53
8	19.79	1098	Linalool	1.03
9	23.54	1174	Menthol (neo iso)	0.04
10	25.74	1220	Methyl chavicol	85.19
11	26.09	1227	Fenchyl acetate (endo)	0.19
12	26.91	1245	Carvone	0.36
13	28.74	1284	Iso bornyl acetate	0.16
14	31.92	1355	Eugenol	0.7
15	33.44	1390	β -Elemene	0.18
16	33.89	1400	Methyl eugenol	0.25
17	34.62	1418	Caryophyllene	0.42
18	35.34	1435	Trans-alpha bergamotene	1.18
19	36.04	1452	α -Humulene	0.11
20	37.28	1482	E- β -Farnesene	0.09
21	38.49	1515	γ -cadinene	0.05
22	38.83	1520	Selinene (7-epi-alpha)	0.19
23	41.20	1580	Caryophyllene oxide	0.49
24	42.42	1612	Cadin-4-en-7-ol (cis)	0.2
25	43.41	1640	Cadinol (epi-alpha)	0.24
			Monoterpenes	1.47
			Oxygenated monoterpenes	91.88
			Sesquiterpenes	2.22
			Oxygenated sesquiterpenes	0.93
Total				96.50

In a previous report, the essential oil of *Ocimum basilicum* contained linalool (69%), eugenol (10%), E- α -bergamotene (3%) (Keita *et al.*, 2000). In another study, the largest components were found to be methyl eugenol (78.02%), α -cubebene (6.17%) and nerol (0.83%) (Özcan and Chalchat, 2002), methyl chavicol (93.0%) (Viyoch *et al.*, 2006). Lee *et al.* (2005) reported linalool (3.94 mg g⁻¹), estragole (2.03 mg g⁻¹) and methyl cinnamate (1.28 mg g⁻¹), and Klimánková *et al.* (2008) reported Linalool, methyl chavicol and eugenol as the major compound. The differences might be due to difference in temperature and topology, as environmental factors, and different growth stages (Goze *et al.*, 2009).

The results of measured antioxidant capacity and antibacterial activities are shown in Table 2. In the method of β -carotene bleaching, the formation of hydroperoxides during linoleic acid oxidation and absence of antioxidants, the yellow color of β -carotene was destroyed, which can be measured spectrophotometrically (Deba *et al.*, 2008). The results showed that the essential oil of *Ocimum basilicum* inhibited oxidation of linoleic acid but its antioxidant capacity was less than BHT. The antioxidant properties of the plant are mostly due to the content of phenolic compounds that can give a hydrogen atom to the free radicals and inhibit oxidation of lipids, as a big problem in food processing. It may also prevent injury to cells (Deba *et al.*, 2008; Wang *et al.*, 2010). Bunrathep *et al.* (2007) showed that *Ocimum basilicum* essence with high contents of methyl chavicol (92.48%) had no antioxidant activity. The essence of *Ocimum basilicum* in the present study had high level

of monoterpene (93.354%), especially methyl chavicol (85.19%) with low levels of eugenol (0.7%) and phenolic compounds (18.1 mg GAEs g⁻¹), which may have antioxidant activity. A previous study demonstrated that *Ocimum basilicum* extracts were as a non toxic substance and oral LD50 was greater than 5,000 mg kg⁻¹. Also, consumption of 5–1,000 mg kg⁻¹ body weight *Ocimum basilicum* essential oil demonstrated no abnormalities in rats (Rasekh *et al.*, 2012).

Reports have shown that gram-negative bacteria are more sensitive than gram-positive bacteria because different compounds of the essence effect on permeability of outer layer in gram-negative bacteria (Pripdeevech *et al.*, 2010). Also, the antibacterial activity of essential oils is related to the monoterpene components, which may inhibit cellular respiratory and transmission of ions to bacteria. In addition, sesquiterpene components have a defense role in plant (Deba *et al.*, 2008).

Table 3 shows the mean TBARS values for raw beef burgers with different concentration of basil essential oil (BEO) during refrigerated storage (4 \pm 1°C) for 12 days. The control sample showed the highest TBARS values. After one day, in the BHT (butylated hydroxytoluene) 0.25% treatment, TBARS values was lower than the control and other samples treatments. On other days (3- 12), BHT 0.25% showed lower oxidation values than the control group (P< 0.05). Also, no statistically significant differences (P> 0.05) were observed between the control sample and the TBARS values BEO25%, BEO 0.125% and BEO 0.062% treatment samples.

Table 2. Antioxidant capacity (AC), total phenol (TP) and antibacterial activity (AA) against *Staphylococcus aureus* of *Ocimum basilicum* essential oil.

	AC (%)	TP (mg GAEs gr ⁻¹)	AA	
			MIC ^a (%)	MBC ^b (%)
<i>Ocimum basilicum</i> essential oil	51.1 \pm 3.8	18.1	1.56	6.25
BHT	90.6 \pm 3.30	-	-	-

^a The Minimum Inhibitory Concentration (MIC), ^b The Minimum Bactericidal Concentration (MBC).

**Table 3.** Mean TBARS values (mg of malonaldehyde kg⁻¹ muscle) for raw beef burger with different concentration of basil essential oil (BEO) during refrigerated storage (4±1°C) for 12 days.^a

Treatment	Days					
	0	1	3	6	9	12
Control	0.95±0.02 ^a	1.04±0.04 ^a	1.36±0.01 ^a	1.92±0.02 ^a	2.57±0.02 ^a	3.22±0.02 ^a
BHT 0.25%	0.95±0.02 ^a	0.97±0.01 ^a	1.12±0.02 ^b	1.51±0.02 ^b	2.14±0.01 ^b	2.86±0.01 ^b
BEO 0.0625%	0.95±0.02 ^a	1.06±0.01 ^a	1.33±0.01 ^a	1.89±0.01 ^a	2.53±0.01 ^a	3.21±0.01 ^a
BEO 0.125%	0.95±0.02 ^a	1.05±0.01 ^a	1.31±0.01 ^a	1.85±0.01 ^a	2.49±0.01 ^a	3.16±0.01 ^a
BEO 0.25%	0.95±0.02 ^a	1.05±0.02 ^a	1.27±0.01 ^b	1.76±0.01 ^a	2.25±0.01 ^a	2.92±0.02 ^a

^a The different superscripts within the same column are significantly different with control group (P< 0.05).

Table 4. Effect of different concentration of basil essential oil (BEO) on *Staphylococcus aureus* in raw beef burger during refrigerated storage (4±1°C) for 12 days.^a

Treatment	Days					
	0	1	3	6	9	12
Control	1.5×10 ³ ± 48 ^a	1.2×10 ³ ± 57 ^a	1.7×10 ³ ± 57 ^a	< 100 ^a	< 100 ^a	< 100 ^a
BEO 0.0625%	1.5×10 ³ ± 48 ^a	1 × 10 ² ± 1 ^a	< 100 ^b	< 100 ^a	< 100 ^a	< 100 ^a
BEO 0.125%	1.5×10 ³ ± 48 ^a	< 100 ^b	< 100 ^b	< 100 ^a	< 100 ^a	< 100 ^a
BEO 0.25%	1.5×10 ³ ± 48 ^a	< 100 ^b	< 100 ^b	< 100 ^a	< 100 ^a	< 100 ^a

^a The different superscripts within the same column are significantly different than the control group (P< 0.05).

Lipid oxidation increased over time in all samples. Increase of TBA values may be caused by the auto oxidation of meat lipids, bacteriological and oxidative rancidity (Salem et al., 2010). The antioxidant activity of medicinal plants and spice essential oils as basil is demonstrated (Rafieian-Kopaie and Baradaran, 2013; Pripdeevech et al., 2010). Activity of EOs is due to their composition as phenolic and flavonoid compounds that can scavenge free radicals, chelate metallic ions, and donate hydrogen in oxidation reactions (Viuda-Martos et al., 2010).

The time-related survival of *S. aureus* following treatment with different concentrations of BEO is demonstrated in Table 4. The BEO in storage temperature (4±1°C) decreased the growth rate of *S. aureus* in beef burger (P< 0.05).

Staphylococcus aureus is a food borne pathogen and can cause a disease transmissible by inappropriate handling and storage of food contaminated with staphylococci; in many countries, it is as the third most common pathogen responsible for

outbreaks of food poisoning (De Souza et al., 2009).

The counted number of bacteria showed a decrease in the count of *S. aureus* in all of the essential oil concentrations. In beef burgers after 24 hours, the viable cell counts decreased by a 2 log cfu g⁻¹ at 4°C, though the number of bacteria in the control was approximately constant (3 log cfu g⁻¹). These results show that BEO has an antibacterial activity against *S. aureus*. After six days of storage, the control samples revealed decrease of *S. aureus* counts. The reason for the decrease in the counts of *S. aureus* may be due to the sensitivity of this bacterium to temperature of refrigerator (4±1°C). Previous works showed similar results about the effects of medicinal plant extracts and essential oils on *S. aureus* in several food models (Jagadeesh Babu et al., 2012; Choobkar et al., 2010; Mahdavian Mehr et al., 2010).

Figure 1 shows the overall acceptability values for cooked beef burgers samples treated with BEO up to 12 days. The results of organoleptic evaluation demonstrated that

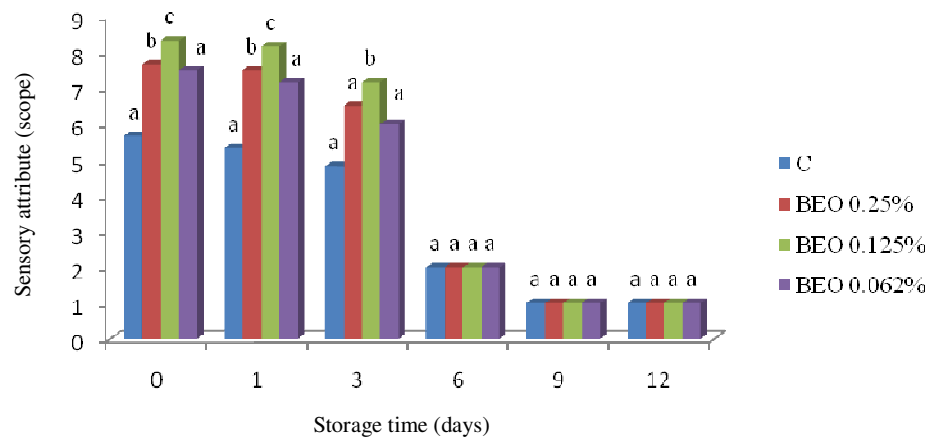


Figure 1. Overall acceptability evaluation of cooked beef burger mixed with different concentration of basil essential oil (BEO).

the best overall acceptability belonged to BEO 0.125% treated samples ($P < 0.05$).

Thus, the significant improvement of BEO treated samples compared to the control samples was due to addition of basil essential oil and this could be related to its aromatic compounds (Kassem *et al.*, 2011).

Furthermore, after the third day of storage, all sensory attributes of the control samples declined, while BEO treated samples were assessed with attribute scores between 5.5 and 7.5. After the sixth day of storage, all of the samples revealed reduction of overall acceptability values.

CONCLUSIONS

With reference to the antioxidant and antibacterial activities of the essential oil of basil and its wide use in the food flavoring and preservation industries, the present results offer the use of BEO along with other natural preservatives in beef burger products.

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فعالیت آنتی اکسیدانی و ضدباکتریایی اسانس ریحان (*Ocimum basilicum* L.) بر روی بیف برگر

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چکیده

هدف از این مطالعه بررسی ترکیبات شیمیایی اسانس، خواص آنتی اکسیدانی و ضدباکتریایی اسانس ریحان در شرایط آزمایشگاهی بود. همچنین میزان اکسیداسیون لیپیدی اسانس در گوشت و اثر ضد باکتریایی آن در فراورده همبرگر مورد بررسی قرار گرفت. در این مطالعه تجربی، اسانس گیاه ریحان به روش تقطیر با بخار آب استخراج شد. سپس میزان و نوع ترکیبات اسانس توسط دستگاه گاز کروماتوگرافی با ردیاب یونیزاسیون شعله و ردیاب جرمی شناسایی شد. سپس تاثیر غلظت های مختلف اسانس ها (صفر، ۰/۰۶۲، ۰/۱۲۵ و ۰/۲۵ درصد) در دمای یخچالی (4 ± 1 درجه سانتیگراد) بر روی اکسیداسیون لیپیدی، بر روی رشد استافیلوکوکوس ارئوس و خواص حسی همبرگر در مدت زمان نگهداری معین (روز صفر، ۱، ۳، ۶، ۹ و ۱۲) مورد بررسی قرار گرفت. بیشترین ترکیبات شناسایی شده در اسانس ریحان متیل کاویکول (۸۵/۱۹٪)، او۱ سینئول (۳/۹۶٪)، ترانس آلفا برگاموتن (۱/۱۸۵٪) و لینالول (۱/۰۳٪) بود. اسانس ریحان در دمای یخچالی سبب کاهش رشد استافیلوکوکوس ارئوس در همبرگر شد ($P < 0/05$). همچنین در میزان پذیرش کلی افزودن اسانس ریحان با غلظت ۰/۱۲۵ درصد به همبرگر کیفیت بهتر حسی ایجاد کرد ($P < 0/05$). با این حال اسانس ریحان اثر معنی داری در کاهش اکسیداسیون لیپیدی در همبرگر های خام نداشت ($P > 0/05$). بنابراین می توان این اسانس را به عنوان یک ترکیب ضد باکتریایی و افزایش دهنده طعم در فراورده های گوشتی مانند همبرگر مورد استفاده قرار داد.