Short Paper

Antimicrobial activities of oregano and nutmeg essential oils combined with emulsifier/stabilizer compound in ready-to-cook barbecued chicken

Shekarforoush, S. S.[1*]; Firouzi, R.[2] and Kafshdozan, K.[3]

[1]Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [2]Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [3]Graduated from School of Veterinary Medicine, Shiraz University, Shiraz, Iran

[*]***Correspondence:*** *S. S. Shekarforoush, Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. E-mail: shekar@shirazu.ac.ir*

(Received 26 Mar 2013; revised version 5 Feb 2014; accepted 26 Feb 2014)

Summary

The effects of essential oils (EOs) of oregano and nutmeg with and without commercial emulsifier/stabilizer compound (E/S) on the microbial quality of ready-to-cook barbecued chicken were evaluated. Barbecued chicken was traditionally prepared. 3 μL/g and 10 mg/g of EOs and/or E/S were then added to the barbecued chicken, respectively. The samples were stored at 3°C for 144 h, 8 and 20ºC for 72 h, accordingly, prior to being subjected to enumeration of aerobic mesophilic and psychrotrophic bacteria at different storage times. No inhibitory effects were detected in the presence of nutmeg EO together with and/or without E/S on the psychrotrophic and aerobic mesophilic counts (AMC) in barbecued chicken. Whereas, AMC and psychrotrophic counts in the samples treated with oregano EO, stored at 8 and 20°C were not dramatically changed. Even though, in the case of treatment with oregano EO and E/S, stored at 3, 8 and 20°C AMC and psychrotrophic counts were significantly affected. Oregano EO was an active antibacterial component, used in combination with commercial E/S, compared to its single use. It can be suggested that using E/S and EO in combination is more likely able to emulsify antimicrobial EO substances and thus increase the efficacy of such substances.

Key words: Oregano, Nutmeg, Barbecued chicken, Emulsifier/stabilizer

Introduction

Traditional Iranian barbecued chicken, a very popular food, is prepared not only in homes and restaurants, but also used on a large scale by food processing industries for ready-to-cook meat. Poultry meat is a highly perishable food commodity providing an almost perfect medium for microbial growth (Desrosier, 1970) and thus various preservation techniques have been developed and adopted for its successful preservation (Lee *et al*., 1996).

One of the spices with proven antimicrobial effects is oregano (*Origanum vulgare*), which has been demonstrated to inhibit the growth of several food borne pathogenic bacteria (Chorianopoulos and Kalpoutzakis, 2004; Burt *et al*., 2005). The antimicrobial activity of oregano has been attributed mainly to the presence of volatile compounds found in its essential oil (EO), especially carvacrol and thymol (Azeredo, 2011).

Nutmeg contains about 10% EO, which is mostly composed of borneol, geraniol, linalool, terpineol, eugenol, myristicin, safrol, camphene, dipentene and pinene (Tainter and Grenis, 2001). The EO of nutmeg has bactericidal effects against several food borne bacteria (Firouzi *et al*., 2007; Shekarforoush *et al*., 2007).

In particular, the inhibitory effects of spices EO against food borne pathogens and spoilage bacteria have been reported extensively (Toroglu, 2011; Raybaudi-Massilia *et al*., 2012). However, there is a limitation to the potential application of EOs in foods since their effectiveness as preservatives has generally been found to decrease significantly when they are tested in real foods as opposed to broths (Nychas, 1995). This reduction has been attributed to the high protein and fat contents of some food products, which can mask the antimicrobial effect of EOs (Shelef, 1983).

Our previous studies showed that some spice extracts do not have any inhibitory effects against bacteria in food (Firouzi *et al*., 2007; Shekarforoush *et al*., 2007). However, they have produced some antibacterial activity *in vitro* and thus we have already suggested that some food components such as oil may absorb the extract and so diminish its antibacterial properties.

Emulsifiers are substances which make it possible to form or maintain a homogenous mixture of two or more immiscible phases such as oil and water in a foodstuff. Stabilizers include substances which enable the maintenance of a homogenous dispersion of two or more immiscible substances in a foodstuff. The purpose of these food additives is to maintain consistent texture and to prevent the separation of ingredients in foodstuff. Any recipe that requires the mixing of ingredients that normally do not mix, such as fat and water, need emulsifiers and stabilizers to impart and maintain the

desired consistency.

In this study, the effect of oregano and nutmeg EOs in combination with emulsifier-stabilizer compound (E/S) on total mesophilic and psychrotrophic bacteria of ready-to-cook barbecued chicken was investigated.

Materials and Methods

Essential oils (EOs)

The pure EOs of oregano (Density: 0.960 at 20°C, GC-MS tested, origin: Bulgaria, steam distillation extraction) and nutmeg (Density: 0.913 at 20°C, GC-MS tested, origin: Indonesia, steam distillation extraction) were obtained from Kobashi Co. (Ide, Devonshire, UK).

Emulsifier-stabilizer compound (E/S)

A commercial E/S compound (Panisol®) containing mono and diglycerides of fatty acids, cellulose, guar gum and carrageenan was obtained from Danisco, Denmark.

Preparation of ready-to-cook barbecued chicken

The barbecued chicken was prepared as described by local chicken meat processing industry. All necessary ingredients including chicken breast, onion, red pepper, lemon juice, saffron, sunflower oil and salt were purchased from the local market.

According to the recipe, the amount of each ingredient used for 1000 g of cubed chicken breast were as follows: salt (4.7 g), red pepper (1.4 g), lemon juice (47 ml), chopped onion (47 g), saffron (0.1 g) and sunflower oil (20 ml). The ingredients were mixed thoroughly and added to the cubed chicken breast. EOs (3 µl g^{-1}) and/or E/S (10 mg g^{-1}) were then added to the barbecued chicken. The control samples were made similarly, except for adding EOs and/or E/S.

Experimental design

The final product was split into units of 10 g, placed in sterile stomacher bags and stored at 3 ± 0.5ºC, 8 ± 0.5ºC and 20 ± 0.5ºC, respectively. Samples subjected to bacteriological analysis were kept at the following incubation times: 3°C for 0, 48, 96 and 144 h; 8 and 20ºC for 0, 24, 48 and 72 h.

Bacteriological analysis

Each sample was diluted in 90 ml of 0.1% buffered peptone water and homogenized for 2 min using stomacher. The homogenate was then ten-fold serially diluted in the 0.1% buffered peptone water. 0.1 ml of aliquot was subsequently surface plated in duplicate using plate count agar (Merck, Germany). All colonies were finally enumerated after incubation for 24-48 h at 37ºC for AMC and 72 h at 20°C for psychrotrophic bacteria (Shekarforoush *et al*., 2007).

Statistical analysis

For each condition, three independent replicates of the experiment were carried out. Before statistical analysis, the data were converted into the logarithmic values of the colony forming units (log CFU g^{-1}) and analyzed using the general linear model procedure of the SPSS, version 11.5 (SPSS, Chicago, Ill.). Duncan's multiple range test was used to determine if any significant difference existed among logs CFU g^{-1} of bacteria.

Results

E/S was not significantly active against psychrotrophic counts and AMC in the experimental groups compared to the control (P>0.05).

Nutmeg EO synergistically with and without E/S was not effective on the growing rates of psychrotrophic bacteria and AMC in the barbecued chicken stored at 3, 8 and 20°C (P>0.05), (Tables 1 and 2). However, in samples treated with oregano EO and stored at 3°C, the populations of psychrotrophic bacteria and the population of AMC remained on a constant level from 5.59 to 5.38 log CFU g^{-1} and from 5.57 to 5.66 while the population of the control group increased as expected from 5.53 to 5.90 and from 5.62 to 5.90. The inhibitory effect of oregano EO was significant after 144 h (P<0.05,

Table 1: Effect of nutmeg essential oil (EO) and its combination with the emulsifier-stabilizer compound (E/S) on aerobic mesophilic bacteria of ready-to-barbecue chicken meat stored at different temperatures

Storage temperature (°C)	Nutmeg EO (3 µl/g)	E/S (10 mg/g)	Mean±SD total viable mesophilic bacteria (Log CFU/g)					
			0 h	24 h	48 h	72 h	96 h	144 h
3	-	-	5.68±0.36		5.63±0.36		5.63±0.34	5.83±0.40
	+	-	5.45±0.20		5.64±0.26		5.68±0.29	5.92±0.19
	-	+	5.51±0.28		5.51±0.34		5.52±0.34	5.93±0.52
	+	+	5.38±0.10		5.31±0.19		5.42±0.16	5.79±0.25
8	-	-	5.45±0.42	5.63±0.51	6.40±0.10	7.22±0.67		
	+	-	5.22±0.60	5.76±0.24	6.23±0.05	6.62±0.19		
	-	+	5.86±0.31	6.11±0.51	6.65±0.49	7.04±0.38		
	+	+	5.62±0.25	5.74±0.33	5.98±0.20	6.33±0.31		
20	-	-	5.17±0.11	5.68±0.15	7.83±0.13	8.35±0.11		
	+	-	5.06±0.04	5.78±0.11	7.49±0.07	7.77±0.18		
	-	+	5.21±0.07	7.34±0.11	8.30±0.07	8.36±0.06		
	+	+	5.23±0.18	6.38±0.03	7.28±0.11	7.56±0.06		

Number of sample examined in each treatment = 3

Table 2: Effect of nutmeg essential oil (EO) and its combination with the emulsifier-stabilizer compound (E/S) on psychrotrophic bacteria of ready-to-barbecue chicken meat stored at different temperatures

Storage temperature (°C)	Nutmeg EO (3 µl/g)	E/S (10 mg/g)	Mean±SD total viable psychrotrophic bacteria (Log CFU/g)					
			0 h	24 h	48 h	72 h	96 h	144 h
3	-	-	5.62±0.20		5.52±0.28		5.62±0.22	5.76±0.25
	+	-	5.27±0.13		5.45±0.02		5.81±0.25	5.64±0.20
	-	+	5.42±0.12		5.54±0.40		5.73±0.45	6.23±0.57
	+	+	5.41±0.14		5.39±0.27		5.60±0.13	6.01±0.15
8	-	-	5.28±0.05	5.48±0.10	6.18±0.54	6.65±0.16		
	+	-	5.46±0.25	5.88±0.49	6.30±0.44	6.58±0.63		
	-	+	5.93±0.30	6.23±0.65	6.77±0.55	7.47±0.58		
	+	+	5.77±0.22	5.76±0.23	6.25±0.69	6.54±0.42		
20	-	-	5.01±0.03	6.07±0.05	8.21±0.09	9.42±0.05		
	+	-	4.98±0.11	5.91±0.07	7.93±0.04	9.19±0.07		
	-	+	5.07±0.02	6.87±0.11	8.23±0.04	8.39±0.05		
	+	+	4.91±0.07	6.32±0.17	7.25±0.18	7.96±0.60		

Number of sample examined in each treatment = 3

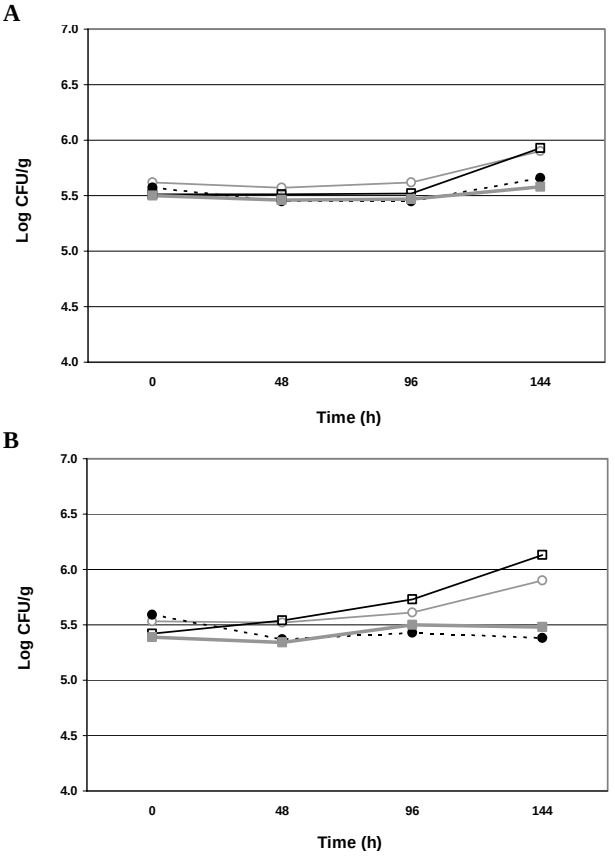

Fig. 1: Growing rates of total viable bacteria in ready-to-cook barbecued chicken affected by oregano essential oil (3 µl g^{-1}) and emulsifier-stabilizer compound (E/S) (10 mg g^{-1}) stored at 3°C. A (mesophilic bacteria), and B (psychrotrophic bacteria). -○- Control, -●- Oregano essential oil, -□- E/S, and -■- Oregano essential oil + E/S

Figs. 1A and B). In addition a significant inhibitory effect was found in samples treated with oregano EO in combination with E/S ($P<0.05$) after 144 h, while E/S alone did not change the population significantly.

In the control group stored at 8°C with and without E/S as well as in samples treated with oregano EO alone and stored at 8°C, a relevant increase in the populations of psychrotrophic bacteria and AMC was observed until the end of the experiment (Figs. 2A and B). However, in the group treated with oregano EO in combination with E/S, a decrease from 5.43 to 5.28 log CFU g^{-1} was found in AMC and only a marginal increase from 5.46 to 5.78 log CFU g^{-1} in the population of psychrotrophic bacteria was observed. The inhibitory effect of oregano EO in combination with E/S on population of psychrotrophic bacteria and AMC was statistically significant ($P<0.01$).

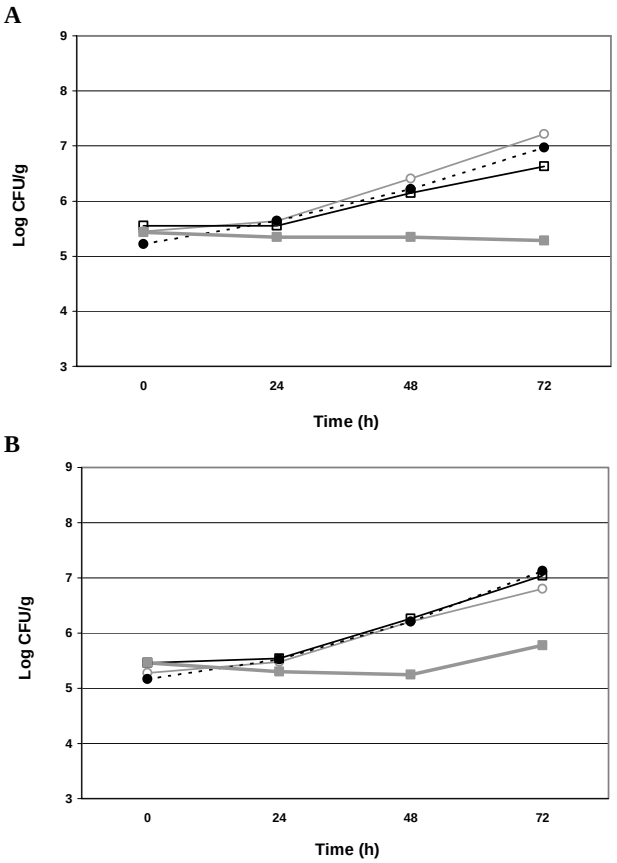

Fig. 2: Growing rates of total viable bacteria in ready-to-cook barbecued chicken affected by oregano essential oil (3 µl g^{-1}) and emulsifier-stabilizer compound (E/S) (10 mg g^{-1}) stored at 8°C. A (mesophilic bacteria), and B (psychrotrophic bacteria). -○- Control, -●- Oregano essential oil, -□- E/S, and -■- Oregano essential oil + E/S

No considerable inhibitory activities in psychotropic counts and AMC were found in samples treated with oregano EO, stored at 20°C (Figs. 3A and B). When oregano EO and E/S were used in combination, a significant inhibition of psychrotrophic counts and AMC was observed ($P<0.01$) compared to oregano alone and to control group (Figs. 3A and B).

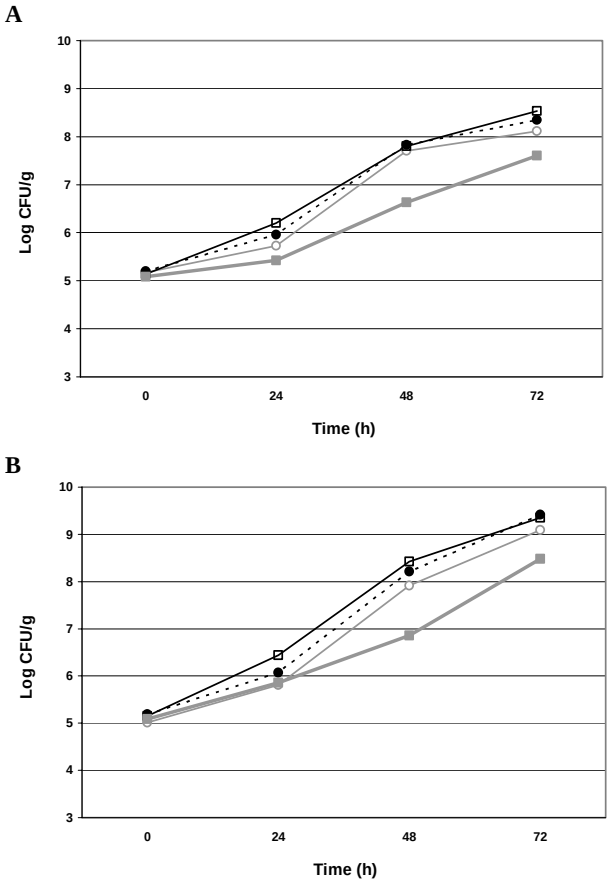

Fig. 3: Growing rates of total viable bacteria in ready-to-cook barbecued chicken affected by oregano essential oil (3 µl g^{-1}) and emulsifier-stabilizer compound (E/S) (10 mg g^{-1}) stored at 20°C. A (mesophilic bacteria), and B (psychrotrophic bacteria). -○- Control, -●- Oregano essential oil, -□- E/S, and -■- Oregano essential oil + E/S

Discussion

In the present study, the antibacterial effect of EOs of nutmeg and oregano combined with E/S in ready-to-cook barbecued chicken was investigated. In our previous studies, we observed the antibacterial activity of EOs of oregano and nutmeg against different genera of bacteria *in vitro* (Shekarforoush *et al*., 2007). However, those studies did not confirm any significant inhibitory effects against *E. coli* O157:H7, *Yersinia enterocolitica* and *Listeria monocytogenes* in ready-to-cook barbecued chicken (Firouzi *et al*., 2007; Shekarforoush *et al*., 2007). It has been shown that the effectiveness of EOs in food may be influenced by the presence of fat, carbohydrate, protein, and salt and also the pH level in food stuffs (Pandit and Shelef, 1994; Tassou *et al*., 1995). Burt *et al*. (2005) showed that stabilizer compounds significantly improved the effectiveness of carvacrol against *E. coli* O157:H7 in broth and they concluded that stabilizer compounds caused delay in the separation of the hydrophobic substrate from the aqueous phase of the medium. The physical structure of a food also may limit the antibacterial activity of EOs (Skandamis *et al*., 2000). Cutter (2000) suggested that antimicrobial activity associated with herbal extracts may be diminished by the presence of adipose component in ground beef. We have already suggested that proteins and fat may absorb such extracts, and thus interfere with the antimicrobial effects (Shekarforoush *et al*., 2007). In this study, using E/S as food additives lead to the theory that in the presence of E/S, soluble forms of EO in the oils produces a stabilized emulsion, which can improve their exposure to microorganisms.

The EO of oregano alone did not affect the growing rates of bacteria in the barbecued chicken stored at 8 and 20°C. However, an antibacterial activity was seen when EO was treated together with E/S in the barbecued chicken. It can be suggested that using E/S and EO, in combination, might be able to emulsify antimicrobial EO substances and thus, increase the efficacy of such substances.

Finally, the results of our *in vivo* investigation revealed a higher antibacterial effect of oregano EO when used together with an emulsifier/stabilizer compound.

Acknowledgement

This research was financially supported by "Natural Antimicrobials Centre of Excellence" which is gratefully acknowledged.

References

Azeredo, GA; Stamford, TLM; Nunes, PC; Neto, NJG; Oliveira, MEG and Souza, EL (2011). Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. Food Res. Inter., 44: 1541-1548.

Burt, SA; Vlielander, R; Haagsman, HP and Veldhuizen, EJA (2005). Increase in activity of essential oil components carvacrol and thymol against *Escherichia coli* O157:H7 by addition of food stabilizers. J. Food Protect., 68: 919-926.

Chorianopoulos, N and Kalpoutzakis, E (2004). Essential oils of *Satureja*, *Origanum*, and *Thymus* species: chemical composition and antibacterial activities against food borne pathogens. J. Agric. Food Chem., 52: 8261-8267.

Cutter, CN (2000). Antimicrobial effect of herb extracts against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* associated with beef. J. Food Protect., 63: 601-607.

Desrosier, NW (1970). *The technology of food preservation*. 3rd Edn., Westport, Avi Publishing Company Inc., PP: 1-25.

Firouzi, R; Shekarforoush, SS; Nazer, AHK; Broumand, Z and Jooyandeh, AR (2007). Effects of essential oils of oregano and nutmeg on growth and survival of *Yersinia*

enterocolitica and *Listeria monocytogenes* in barbecued chicken. J. Food Protect., 70: 2626-2630.

Lee, M; Sebranek, JG; Olson, DG and Dickson, JS (1996). Irradiation and packaging of fresh meat and poultry. J. Food Protect., 59: 62-72.

Nychas, GJE (1995). Natural antimicrobials from plants. In: Gould, GW (Ed.), *New methods of food preservation*. London, Blackie Academic Professional. PP: 58-89.

Pandit, VA and Shelef, LA (1994). Sensitivity of Listeria monocytogenes to rosemary (*Rosmarinus officinialis* L.). Food Microbiol., 11: 57-63.

Raybaudi-Massilia, R; Zambrano-Duran, A; Mosqueda-Melgar, J and Calderon-Gabaldon, MI (2012). Improving the safety and shelf-life of orange and mango juices using Panax ginseng, malic acid and potassium sorbate. J. Verbrauch. Lebens., 7: 272-282.

Shekarforoush, SS; Nazer, AHK; Firouzi, R and Rostami, M (2007). Effect of storage temperatures and essential oils of oregano and nutmeg on the growth and survival of *Escherichia coli* O157:H7 in barbecued chicken used in Iran. Food Control. 18: 1428-1433.

Shelef, LA (1983). Antimicrobial effects of spices. J. Food Safety. 6: 29-44.

Skandamis, P; Tsigarida, E and Nychas, GJE (2000). Ecophysiological attributes of *Salmonella typhimurium* in liquid culture and within a gelatin gel with or without the addition of oregano essential oil. World J. Microbiol. Biotech., 16: 31-35.

Tainter, DR and Grenis, AT (2001). *Spices and seasonings*. 2nd Edn., New York, John Wiley and Sons. PP: 34-159.

Tassou, CC; Drosinos, EH and Nychas, GJE (1995). Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4°C and 10°C. J. Appl. Bacteriol., 78: 593-600.

Toroglu, S (2011). *In-vitro* antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. J. Environ. Biol., 32: 23-29.