ANTIMICROBIAL ACTIVITIES OF OREGANO ESSENTIAL OIL COMBINED WITH EMULSIFIER/STABILIZER COMPOUND IN READY-TO-COOK BARBECUED CHICKEN

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

[1] Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, 71345-1731, Iran
[2] Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, 71345-1731, Iran
[3] Graduated from the School of Veterinary Medicine, Shiraz University, Shiraz, 71345-1731, Iran
*Corresponding author (phone: +98-711-2286950; fax: +98-711-2286940; e-mail: shekar@shirazu.ac.ir)

Abstract-- The effects of essential oil (EO) of oregano accompany 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. Three µL g^{-1} and 10 mg g^{-1} of EO and/or E/S were then added to the ready-to-cook barbecued chicken, respectively. The samples were stored at 3 ºC for 144 h, 8 ºC and 20 ºC for 72 h. Aerobic plate count (APC) in the samples, stored at 3 °C, 8 °C and 20 °C were significantly affected. Oregano EO was an active antibacterial component, using in combination with commercial E/S, compared to its single use. It can be suggested that using E/S and EO, in combination, more likely is able to emulsify antimicrobial EO substances and thus increase the efficacy of such substances.

Index Terms-- **Chicken, Emulsifier/stabilizer, Oregano**

I. INTRODUCTION

For a very long time, spices and herbs have been added to foods mainly as seasoning additives due to their aromatic characteristics. However it has also been claimed that they can preserve many foods (Nychas, 1995). 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 (Burt, Vlielander, Haagsman & Veldhuizen,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 (Skandamis & Nychas, 2000).

In particular, the inhibitory effects of spices EO against food borne pathogens and spoilage bacteria have been reported extensively (Quattara, Simard, Holley, Piette & Begin, 1997; Tassou, Drosinos & Nychas, 1996). 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 have not had any inhibitory effects against bacteria in food. 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.

In this study, the effect of oregano EO in combination with emulsifier-stabilizer compound (E/S) on aerobic plate count (APC) of ready-to-cook barbecued chicken was investigated.

II. MATERIALS AND METHODS

A. EO of oregano

The pure EO of oregano (Density: 0.960 at 20 °C, GC-MS tested, origin: Bulgaria, steam distillation extraction) was obtained from Kobashi company (Ide, Devonshire, UK).

B. E/S compound

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

C. Preparation of barbecued chicken

The barbecued chicken was prepared as it was described by a 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 ingredients used for 1000 grams of cubed chicken breast were as follows: salt (4.7 g), red pepper (1.4 g), lemon juice (47 ml), chopped onion (47g), saffron (0.1 g) and sunflower oil (20 ml). The ingredients were mixed thoroughly and added to the cubed chicken breast. EO (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 EO and/or E/S.

D. Experimental design

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

E. 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 aliquots were subsequently surface plated in duplicate using plate count agar (Merck). All colonies were finally enumerated after incubation for 24-48 h at 37 ºC.

F. Statistical analyses

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.

III. RESULTS AND DISCUSSION

Samples stored at 3 °C, similar changes in APC were observed in the treatment groups in comparison to the control samples ($p>0.05$, Fig. 1), while when we stored the samples at 8 °C, APC of the control group was increased from 4.95 to 6.72 log CFU g^{-1} during the storage time. APC in the samples treated with oregano EO and E/S were increased from 5.36 to 6.54 and 4.72 to 6.47 log CFU g^{-1}, respectively. Interestingly, these finding in the samples treated with oregano EO+E/S were decreased from 5.20 to 4.85 log CFU g^{-1} which was significant compared to the other groups ($p= 0.001$, Fig. 2).

No considerable activities were found against APC in the samples treated with oregano EO and E/S, stored at 20 °C. But in the presence of both components (oregano EO and E/S), the APC was changed from 5.08 to 7.46 log CFU g^{-1} during the storage time which was significant ($p= 0.001$, Fig. 3).

In our previous studies, we observed that the antibacterial activity of EOs of oregano against different genera of bacteria *in vitro*. 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, Shekarforoush, Nazer, Broumand & Jooyandeh, 2007; Shekarforoush, Nazer, Firouzi & Rostami, 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 & Shelef, 1994; Tassou, Drosinos & Nychas, 1995*)*. Burt, Vlielander, Haagsman and Veldhuizen (2005) showed 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, Tsigarida & Nychas, 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. In this study, using E/S as food additives lead to a theory that in the presence of E/S, soluble forms of EO in the oils, make a stabilized emulsion which can improve their exposure to microorganisms.

IV. CONCLUSION

The results of our *in vivo* investigation revealed that oregano EO was not significantly active against APC in ready-to-cook barbecued chicken. However, a higher antibacterial effect of oregano EO was seen when we used it in conjunction to an emulsifier/stabilizer compound.

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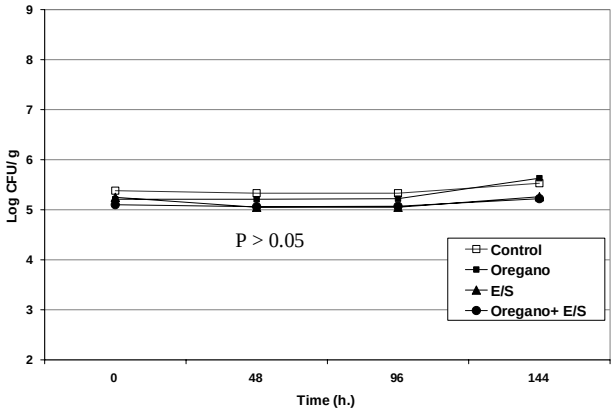

FIGURE 1. Aerobic plate count in ready-to-cook barbecued chicken affected by oregano essential oil (3 µl g^{-1}), emulsifier-stabilizer compound (E/S) (10 mg g^{-1}) and oregano essential oil + E/S stored at 3 °C.

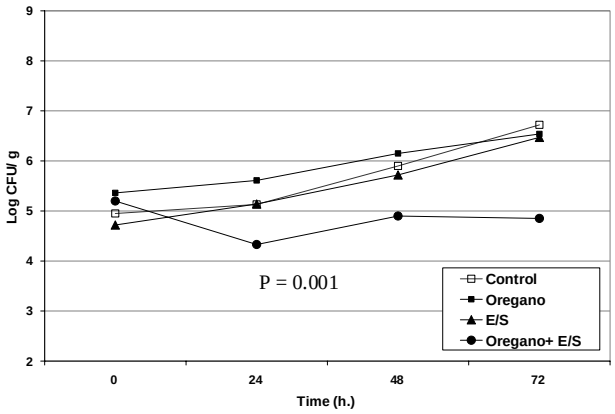

FIGURE 2. Aerobic plate count in ready-to-cook barbecued chicken affected by oregano essential oil (3 µl g^{-1}), emulsifier-stabilizer compound (E/S) (10 mg g^{-1}) and oregano essential oil + E/S stored at 8 °C.

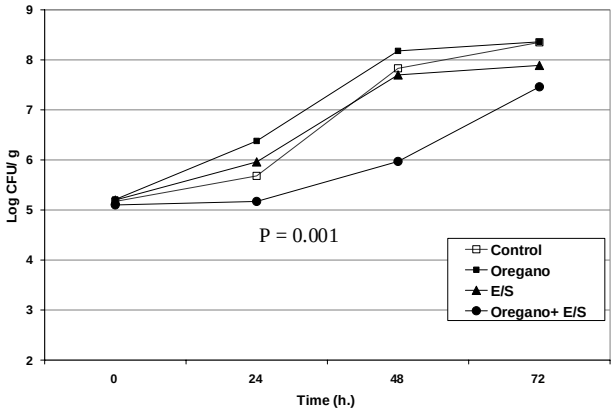

FIGURE 3. Aerobic plate count in ready-to-cook barbecued chicken affected by oregano essential oil (3 µl g^{-1}), emulsifier-stabilizer compound (E/S) (10 mg g^{-1}) and oregano essential oil + E/S stored at 20 °C.