EFFECT OF *ZATARIA MULTIFLORA* BOISS ESSENTIAL OIL AND GRAPE SEED EXTRACT ON THE SHELF LIFE OF RAW BUFFALO PATTY AND FATE OF INOCULATED *LISTERIA MONOCYTOGENES*

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ABSTRACT

The present study was conducted to investigate the effect of *Zataria multiflora* Boiss essential oil (ZEO) and grape seed extract (GSE) on microbial and chemical changes in raw buffalo patty at a temperature of 8C. First, chemical composition and minimum inhibitory concentration of oil against *Listeria monocytogenes* were identified. Then, ZEO and GSE were added in raw buffalo patties and analyzed for spoilage microorganism count, lipid oxidation, pH, sensory attributes and inoculated *L. monocytogenes* survival at 8C for 9 days. Results revealed that samples containing 0.1% ZEO + 0.2% GSE showed significant decrease in the growth of all tested microbial groups as well as the most decrease in the level of lipid oxidation at the end of storage period. Control and samples with 0.1% ZEO were the most preferred samples by sensory panelist, indicating that ZEO and GSE can be practically applied in food systems especially in meat in order to extend the shelf life.

PRACTICAL APPLICATIONS

Buffalo meat is highly prone to microbial and chemical spoilage as they are rich in essential nutrients and putrefying in nature. The adverse effects of various chemical preservatives caused to attract the consumer's attention to natural alternatives. Therefore, in order to increase the shelf life and quality characteristics of meat, new processing technologies and new ingredient systems that are associated with natural and organic foods are applied. These results demonstrate the potential combined use of grape seed extract and *Zataria multiflora* Boiss essential oil to the meat industry in the development of novel healthy meat products with improved shelf life and superior product quality.

INTRODUCTION

Nowadays, safe and healthy meat products with longer shelf life are of great importance for both consumers and food industries. Being a nutrient medium for bacterial growth, ground meat and minced meat products are considered as highly perishable foods. The major factors affecting shelf life of such products are microbial growth and oxidative rancidity (Shan *et al.* 2009).

To extend the shelf life of foods, wide range of natural biopreservatives are used, and therefore, essential oils and various extracts of plants are outstanding substitution for chemical preservatives (Burt 2004; Pajohi *et al.* 2011). Numerous studies indicated antioxidant, antibacterial, antifungal and antiviral properties of essential oils. The effective ability of essential oils at the interface with the lipid- and water-compatible portions of meat is related to partially hydrophobic nature of these components, and several studies have demonstrated the beneficial roles of essential oils applied for food preservations (Shan *et al.* 2009).

Zataria multiflora Boiss belongs to Lamiaceae family that grows in various parts of Asia, especially in Iran, Pakistan

and Afghanistan (Hosseinzadeh *et al.* 2000). Because of the high portion of phenolic compounds such as thymol and carvacrol, *Zataria multiflora* essential oil (ZEO) exhibits antioxidant, antibacterial and antifungal effects (Aliakbarlu *et al.* 2013; Aminzare *et al.* 2014).

Grape seed extract (GSE) is a by-product derived from grape seeds (*Vitis vinifera*), which is a generally recognized as safe product, containing high flavonoid and phenolic content (Over *et al.* 2010). Resveratrol (trans-3, 4′5-trihydroxystilbene), a phenolic compound present in wines and various parts of grape, is responsible for antioxidant and antimicrobial activities of the GSE (Kulkarni *et al.* 2011). Comparison of antioxidant power of resrevatrol with other antioxidants by Murcia and Martinez-Tome showed the following result: butylated hydroxyanisole > resveratrol > propyl gallate > tripolyphosphate > vanillin > phenol > butylated hydroxytoluene > alpha-tocopherol (Murcia and Martínez-Tomé 2001). There are several studies on antimicrobial and antioxidant effect of GSE in ground chicken (Brannan 2009), raw frozen vacuum-packed beef (Rojas and Brewer 2008) and ground beef (Ahn *et al.* 2004).

As essential oils in food products impart strong flavor to food and interact with some food ingredients, using these compounds in combination is considered as a main way to decrease unsatisfactory effect of essential oils in food products (Stojanovic *et al.* 2012). In this case lower concentrations of essential oils can be used and undesirable changes in flavor or aroma do not occur (Burt 2004).

In the present study, (1) phytochemical properties of ZEO; (2) *in vitro* antibacterial activity of ZEO against *L. monocytogenes*; and (3) the effect of ZEO and GSE alone and in combination at lower levels of sensorial threshold on microbial changes (mesophilic and psychrotrophic viable counts, lactic acid bacteria [LAB], *Pseudomonas* spp. yeast and inoculated *L. monocytogenes* count), lipid peroxidation (malondialdehyde measurement), pH values and sensorial characteristics of raw buffalo patties during a 9 day storage time at 8C were investigated.

MATERIALS AND METHODS

Preparation and Chemical Analysis of ZEO

The plant of *Zataria multiflora* Boiss was purchased from local groceries and authenticated at the Faculty of Agriculture and Natural Sciences, Urmia University, Urmia, Iran. Dried aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus according to the European pharmacopeia. The oil was dehydrated with sodium sulfate, filtered by 0.22 μm filters and then stored in colored glass ampoules in the dark at 4C for further analysis (Hashemi *et al.* 2013).

Chemical composition of ZEO was analyzed using a gas chromatograph (Hewlett-Packard, Santa Clara, CA; 6890N) equipped with a column HP-5MS (30 m length × 0.25 mm i.d., film thickness 0.25 mm) coupled to a mass spectrometer (Hewlett-Packard 5973N). The chromatographic conditions were as follows: helium flow rate was 1.5 mL/min; temperature programmed to rise from 40 to 240C with a gradient of 3C/min (holding the initial and final temperature for 6 min); followed by a temperature enhancement of 15C/min up to 300C (holding for 3 min); injector port temperature; and detector temperature were set at 290 and 250C, respectively. The compounds were identified by comparing their retention index with those of authentic samples and mass spectral data available in the library (Wiley 2001 data software) (Aminzare *et al.* 2015).

Preparation of GSE

Commercial GSE powder was obtained from Sigma (St. Louis, MO) and 1% w/v stock solution was prepared in distilled water. The solution was subsequently sterilized by filtration via 0.22 μm filters.

Determination of Minimum Inhibitory Concentration Value of ZEO

The minimum inhibitory concentration (MIC) against *L. monocytogenes* (ATCC19118) was measured by broth microdilution method (Aliakbarlu *et al.* 2013; Hashemi *et al.* 2013). First, *L. monocytogenes* was adjusted to 10^5 colony-forming unit (cfu)/mL in Tryptic soy broth, and ZEO was dissolved in dimethyl sulfoxide (10%). Then, serial twofold dilution of the essential oil (5,000–156 μg/mL) and bacterial suspension were inoculated to the 96-well microplates, and the absorbance was measured at 600 nm by a microplate reader (Biotek Instrument Inc., Winooski, VT). Microplates incubated at 37C for 24 h and after incubation turbidity in the wells visually observed, and absorbance was measured by microplate reader. The MIC was defined as the lowest concentration of the ZEO showing a complete growth inhibition of the tested microorganisms (Fu *et al.* 2007).

Determination of Appropriate Level of ZEO

Post-rigor buffalo muscles were purchased directly from a local meat packaging plant and while refrigerated transported immediately to the laboratory. Meat was ground in a meat grinder under an aseptic condition. Ground meats with different concentrations of ZEO (0, 0.05, 0.1, 0.25 and 0.5% v/w) were homogenized in a mixer. In order to form raw patties, a burger mold was used and sensory quality of

the samples (color and odor) was evaluated by a 10 member semi-trained panel from the Department of Food Hygiene and Quality Control, Urmia University, Urmia, Iran.

Shelf Life Study

Preparation of Meat Patties Containing ZEO and GSE. In preparation of treated meat patties, results of initial sensory evaluation of ZEO were used. Among different concentrations of ZEO (0, 0.05, 0.1, 0.25 and 0.5% v/w), concentration of 0.1% was the most preferred of the treatments showing acceptable sensory scores for odor and color attributes. Based on the results of initial sensory evaluation and MIC determination, 0.1% ZEO was applied in further evaluations in buffalo patty. The concentrations of GSE were established 0.1 and 0.2% according to the results of previous studies (Jayaprakasha *et al.* 2003; Brannan 2009; Over *et al.* 2010). ZEO and GSE were added either alone or in combination with the samples using a micropipette and were divided into six groups: (1) control (no addition); (2) 0.1% ZEO; (3) 0.1% GSE; (4) 0.2% GSE; (5) 0.1% ZEO + 0.1% GSE; and (6) 0.1% ZEO + 0.2% GSE. After appropriate homogenization, patties were formed using a burger mold and were separately packaged in sterile polyethylene bags (Zipack, Tehran, Iran) and stored in temperature-controlled laboratory refrigerator (8C) before analysis at 0, 3, 6 and 9 days.

Microbial Evaluation. Samples were aseptically taken and homogenized with sterile peptone water. After decimal dilutions, 0.1 mL of each dilution was spread on specific culture media such as plate count agar (PCA; Merck, Darmstadt, Germany) for total mesophilic viable count (TMVC) at 35C for 24 h; PCA for total psychrotrophic viable count (TPVC) at 7C for 5–7 days; *Pseudomonas* agar base (Oxoid, Hampshire, UK) supplemented with cetrimide, fucidine and cephaloridine for *Pseudomonas* spp. at 25C for 48 h; de Man Rogosa Sharpe agar (Merck, Darmstadt, Germany) for LAB at 30C for 72 h; and Rose Bengal chloramphenicol selective agar (Liofilchem, Roseto degli Abruzzi, Italy) for yeasts at 25C for 3–5 days (Tajik *et al.* 2014).

pH Assay. pH values were measured after homogenizing 10 g of samples with 90 mL distilled water by a pH meter (pH 510 Eutech; CyberScan, Ayer Rajah, Singapore) using the method previously described (Ehsani *et al.* 2014).

Measurement of Lipid Oxidation. Degree of lipid oxidation in samples was analyzed using thiobarbituric acid reactive substances (TBARS) method as described by Tajik *et al.* (2014). In brief, 10 g of every sample was homogenized with 35 mL of cold (4C) extraction solution containing 4% perchloric acid and 1 mL of butylated hydroxy toluene at 13,500 rpm for 1 min. The blended sample was filtered through Whatman no. 1 filter paper into a 50 mL falcon tube. The filtrate was adjusted to 50 mL with 4% perchloric acid and 5 mL of this mixture was added to 5 mL of TBA solution (0.02 mol/L). The final mixture was incubated in a water bath at 100C for 60 min to develop the malondialdehyde–TBA complex. The absorbance at 532 nm was measured after cooling with cold water for 10 min. TBARS values were expressed as mg malondialdehyde per kg sample. TEP (1,1,3,3-tetraethoxypropane) was used for preparation of standard curve (Tajik *et al.* 2014).

Sensory Evaluation. The samples were evaluated by a panel of seven experienced judges (laboratory trained). The judges were not informed about the experimental approach and the samples were blind. Raw patties were evaluated about odor, color and overall acceptability attributes on the basis of the 9-point hedonic scale (9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely) (Ehsani *et al.* 2014).

Survival of *L. monocytogenes* Inoculated in Patties Containing ZEO and GSE

First, to destroy meat surface pathogens and spoilage microorganisms, the meat surface was sprayed with 95% v/v ethanol and then burnt. The burnt sections were removed and sterile tissue was aseptically ground in a meat grinder. In the next step, the treated samples (six groups) were inoculated by 5 $\log_{10}$ cfu/g viable cells of *L. monocytogenes* and stomached at 300 rpm for 30 s. After homogenization, samples were packaged in sterile bags and stored in 8C for up to 9 days.

In order to sample treatments, aliquots of 1 mL from homogenized samples were removed and decimal dilutions (1:10) in 0.1% sterile peptone water solution were prepared and 0.1 mL of each dilution was spread on *Listeria* chrom agar plate (CHROM agar, Paris, France) at 37 ± 1C for 24 h (Tajik *et al.* 2014).

Statistical Analysis

All experiments in this study were carried out in triplicate, and for statistical analysis of data (analysis of variance), SPSS software (version 19) was applied. Multiple comparisons Tukey's test was used at the confidence level of $P < 0.05$ to determine the significant difference among samples.

RESULTS AND DISCUSSION

ZEO Chemical Composition

The chemical composition of essential oil is presented in Table 1. Twenty-nine compounds representing 98.38% of total oil were reported. The major components of essential oil were thymol (64.87%), g-terpinene (9.11%), p-cymene (5.63%), carvacrol (4.65%) and trans-caryophyllene (3.41%). Thymol and carvacrol were identified as the major components of ZEO by other researchers as well (Shafiee and Javidnia 1997; Sharififar *et al.* 2007).

MIC Value of ZEO

According to results obtained, the MIC value of ZEO against *L. monocytogenes* was 625 μg/mL. Aliakbarlu *et al.* (2013) reported that the MIC values of ZEO were 0.625 and 1.25 mg/mL for gram-positive (*B. cereus* and *L. monocytogenes*) and gram-negative (*E. coli* and *S. typhimurium*) bacteria, respectively, which was completely consistent with the current study (Aliakbarlu *et al.* 2013). Rahnama *et al.* (2009) mentioned that the MIC of ZEO against *L. monocytogenes* was 0.0095 mg/mL (Rahnama *et al.* 2009). Differences in characteristics of applied bacterial species and in components of applied ZEO can be the reason of different obtained results for MIC values (Aminzare *et al.* 2014).

TABLE 1. CHEMICAL COMPOSITION OF *ZATARIA MULTIFLORA* BOISS ESSENTIAL OIL

	Compounds	KI*	Area (%)
1	a-Thujene	931	0.57
2	a-Pinene	939	0.88
3	Camphene	953	0.92
4	b-Pinene	980	0.2
5	b-Myrcene	991	1.01
6	a-Phellandrene	1,002	0.15
7	a-Terpinene	1,015	1.63
8	p-Cymene	1,024	5.63
9	Sylvestrene	1,028	0.31
10	1,8-Cineole	1,029	0.8
11	g-Terpinene	1,061	9.11
12	Cis-Sabinene hydrate	1,066	0.51
13	a-Terpinolene	1,088	0.15
14	Borneol	1,163	1.42
15	a-Terpineol	1,209	0.11
16	Thymol	1,300	64.87
17	Carvacrol	1,314	4.65
18	Thymol acetate	1,355	0.08
19	Trans-Caryophyllene	1,422	3.41
20	Aromandrene	1,439	0.18
21	a-Humulene	1,453	0.09
22	g-Muurolene	1,476	0.11
23	Viridiflorene	1,496	0.2
24	Bisabolene	1,507	0.11
25	g-Cadinene	1,513	0.09
26	d-Cadinene	1,522	0.2
27	Spathulenol	1,575	0.1
28	Caryophyllene oxide	1,580	0.34
29	Carvacrol methyl ether	1,242	0.55
Total			98.38%

* Kovats indices calculated against n-alkanes on HP-5 column.

Shelf Life Study

Effect of ZEO and GSE on Spoilage Microorganism Count. Changes in TMVC, TPVC, *Pseudomonas* spp., LAB and yeast populations of experimental buffalo patties, including control, samples with 0.1% ZEO, 0.1% GSE, 0.2% GSE, 0.1% ZEO + 0.1% GSE and 0.1% ZEO + 0.2% GSE, stored at 8C for up to 9 days are presented in Table 2.

The initial mesophilic, psychrotrophic and *Pseudomonas* spp. counts in all samples were in the range of 2.53–3.51, 4.38–5.01 and 3.64–4.12 $\log_{10}$ cfu/g, respectively. On the other hand, the initial LAB and yeast counts were in the range of 2.43–3.09 and 3.29–3.57 $\log_{10}$ cfu/g, respectively. All treatments significantly ($P < 0.05$) decreased the population of TPVC, LAB and yeast as compared with control at the end of storage period. A total of 0.1% ZEO + 0.2% GSE samples significantly ($P < 0.05$) inhibited the growth of *Pseudomonas* spp. as well. However, this treatment also exhibited a potent effect on all other microbial counts such as TPVC, TMVC, LAB and yeast. These results implied that combined use of ZEO and GSE exhibits synergistic effect on the buffalo meat spoilage microorganisms.

The individual EOs and extracts contain complex components that may lead to additive, synergistic or antagonistic effects, when combined together (Fu *et al.* 2007). To the best of our knowledge, there is not any study on synergistic effects of ZEO and GSE on spoilage microorganisms, but results of Tajik *et al.* (2014) on clove oil and GSE were not in agreement with the present study, which is probably attributed to different kinds of used essential oil (clove oil) with GSE (Tajik *et al.* 2014).

A total of 0.1% GSE samples and 0.2% GSE samples exerted similar effect on all microbial groups at the end of storage period and did not inhibit growth of TMVC and *Pseudomonas* spp. during storage. A total of 0.1% ZEO was significantly ($P < 0.05$) more potent than extracts (0.1% GSE and 0.2% GSE) in all microbial counts.

Higher mesophilic, psychotropic and *Pseudomonas* spp. counts compared with LAB and yeast that could be due to low sugar concentration of meat resulted in domination of gram-negative bacteria in meat spoilage. *Pseudomonas* spp. that produce extracellular proteases and lipases at low temperatures therefore cause quality deterioration of meat products. Besides, gram-positive bacteria generally tend to

TABLE 2. ANTIMICROBIAL EFFECTS OF ZEO AND GSE AND THEIR COMBINATION ON DIFFERENT SPOILAGE MICROORGANISMS IN RAW BUFFALO PATTIES DURING STORAGE AT 8C FOR 9 DAYS

Storage time (days)	Control	0.1% ZEO	0.1% GSE	0.1% GSE	0.2% GSE	0.1% ZEO + 0.1% GSE	0.1% ZEO + 0.2% GSE
TMVC							
0	3.26* ± 0.16^{ab}	3.02 ± 0.18^{b}	2.53 ± 0.12^{c}	2.53 ± 0.12^{c}	3.51 ± 0.18^{a}	2.84 ± 0.15^{bc}	2.54 ± 0.2^{c}
3	5.77 ± 0.18^{b}	4.67 ± 0.11^{c}	6.07 ± 0.19^{ab}	6.07 ± 0.19^{ab}	6.24 ± 0.22^{a}	4.74 ± 0.14^{c}	4.72 ± 0.15^{c}
6	7.38 ± 0.25^{a}	6.15 ± 0.11^{b}	7.15 ± 0.22^{a}	7.15 ± 0.22^{a}	7.2 ± 0.18^{a}	5.91 ± 0.09^{b}	5.78 ± 0.21^{b}
9	7.77 ± 0.15^{a}	6.54 ± 0.12^{b}	7.73 ± 0.18^{a}	7.73 ± 0.18^{a}	7.82 ± 0.16^{a}	6.37 ± 0.24^{b}	6.3 ± 0.17^{b}
TPVC							
0	4.75 ± 0.18^{ab}	5 ± 0.13^{a}	4.88 ± 0.09^{a}	4.88 ± 0.09^{a}	4.72 ± 0.15^{ab}	5.01 ± 0.16^{a}	4.38 ± 0.13^{b}
3	5.27 ± 0.06^{b}	5.61 ± 0.03^{a}	4.92 ± 0.08^{d}	4.92 ± 0.08^{d}	5.39 ± 0.04^{b}	5.71 ± 0.06^{a}	5.11 ± 0.03^{c}
6	6.66 ± 0.21^{a}	6.24 ± 0.11^{ab}	6.42 ± 0.12^{a}	6.42 ± 0.12^{a}	6.47 ± 0.18^{a}	6.45 ± 0.19^{a}	5.89 ± 0.16^{b}
9	9.34 ± 0.17^{a}	7.89 ± 0.22^{c}	8.68 ± 0.11^{b}	8.68 ± 0.11^{b}	8.71 ± 0.21^{b}	8.01 ± 0.22^{c}	7.37 ± 0.15^{d}
Pseudomonas spp.							
0	3.85 ± 0.13^{abc}	3.79 ± 0.08^{abc}	3.98 ± 0.19^{ab}	3.98 ± 0.19^{ab}	4.12 ± 0.11^{a}	3.71 ± 0.07^{bc}	3.64 ± 0.11^{c}
3	6.61 ± 0.06^{a}	5.7 ± 0.08^{c}	6.34 ± 0.06^{b}	6.34 ± 0.06^{b}	6.6 ± 0.02^{a}	5.57 ± 0.07^{c}	5.59 ± 0.05^{c}
6	7.1 ± 0.15^{a}	6.18 ± 0.11^{c}	7.16 ± 0.15^{a}	7.16 ± 0.15^{a}	7.15 ± 0.14^{a}	6.9 ± 0.1^{ab}	6.73 ± 0.15^{b}
9	8.8 ± 0.09^{a}	7.77 ± 0.15^{b}	8.89 ± 0.21^{a}	8.89 ± 0.21^{a}	8.71 ± 0.16^{a}	7.35 ± 0.15^{bc}	7.18 ± 0.14^{c}
Lactic acid bacteria							
0	3.09 ± 0.1^{a}	2.79 ± 0.17^{ab}	2.47 ± 0.19^{bc}	2.47 ± 0.19^{bc}	2.43 ± 0.08^{c}	3.05 ± 0.05^{a}	2.86 ± 0.09^{a}
3	5.11 ± 0.1^{a}	4.26 ± 0.2^{c}	4.84 ± 0.15^{ab}	4.84 ± 0.15^{ab}	4.5 ± 0.11^{bc}	4.26 ± 0.09^{c}	5.16 ± 0.11^{a}
6	6.12 ± 0.14^{a}	5.68 ± 0.15^{b}	5.98 ± 0.11^{ab}	5.98 ± 0.11^{ab}	5.75 ± 0.14^{ab}	5.75 ± 0.13^{ab}	5.99 ± 0.15^{ab}
9	6.73 ± 0.16^{a}	5.95 ± 0.19^{b}	6.24 ± 0.22^{ab}	6.24 ± 0.22^{ab}	6.07 ± 0.18^{b}	5.95 ± 0.2^{b}	5.78 ± 0.18^{b}
Yeast							
0	3.43 ± 0.03^{a}	3.29 ± 0.03^{a}	3.50 ± 0.09^{a}	3.50 ± 0.09^{a}	3.57 ± 0.04^{a}	3.45 ± 0.14^{a}	3.73 ± 0.42^{a}
3	4.91 ± 0.12^{a}	3.87 ± 0.09^{c}	4.78 ± 0.12^{ab}	4.78 ± 0.12^{ab}	4.91 ± 0.11^{a}	4.16 ± 0.1^{c}	4.59 ± 0.13^{b}
6	5.46 ± 0.06^{a}	4.20 ± 0.02^{c}	5.23 ± 0.15^{a}	5.23 ± 0.15^{a}	5.28 ± 0.12^{a}	4.57 ± 0.09^{b}	4.67 ± 0.1^{b}
9	6.04 ± 0.1^{a}	5.15 ± 0.07^{b}	5.67 ± 0.18^{ab}	5.67 ± 0.18^{ab}	5.74 ± 0.2^{ab}	5.61 ± 0.11^{ab}	5.74 ± 0.48^{ab}

Notes: All values are mean ± standard deviation of three replicates. Values in the same row with different superscripts mean that the values are significantly different ($P < 0.05$).* Log_{10} colony-forming unit/g.GSE, grape seed extract; TMVC, total mesophilic viable count; TPVC, total psychrotrophic viable count; ZEO, *Zataria multiflora* Boiss essential oil.

be more sensitive to EOs and extracts than gram-negative bacteria (Zhang *et al.* 2009).

pH Measurement. As can be seen in Fig. 1, control and 0.1% ZEO + 0.2% GSE samples had the lowest (5.87) and highest (6.75) pH value at the end of storage time, respectively. The result showed that adding ZEO and GSE exhibited significant effect ($P < 0.05$) on inhibition of pH drop. More pH drop in control than other samples could be due to more growth of LAB that would lead to further reduction

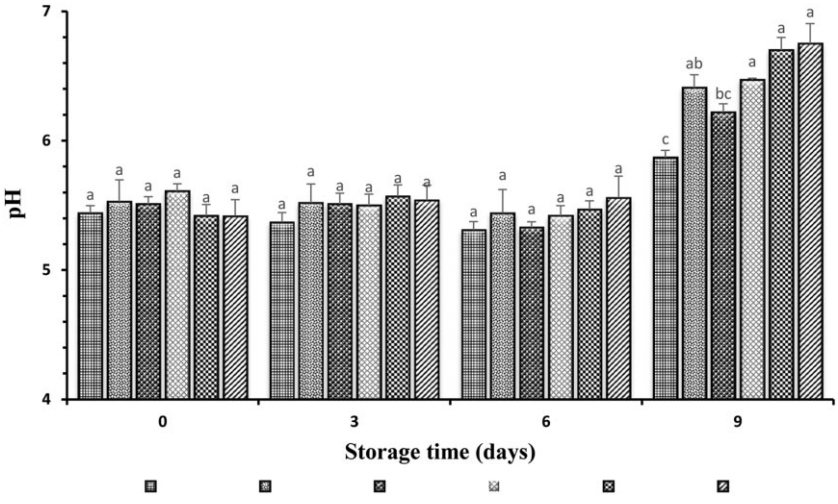

FIG. 1. PH VALUES OF RAW BUFFALO PATTIES CONTAINING DIFFERENT LEVELS OF ZEO AND GSE DURING 9 DAYS OF STORAGE AT 8C.
Values with different lowercase letters are significantly different ($P < 0.05$)

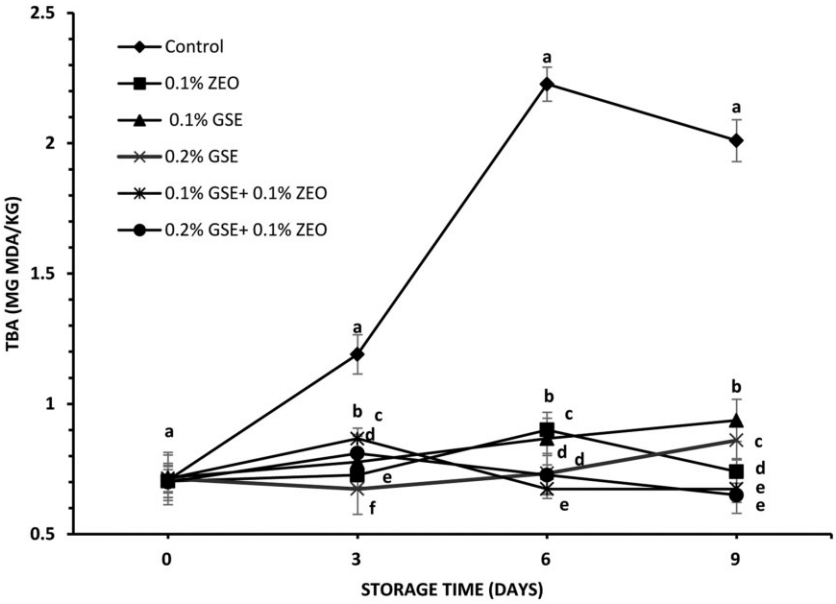

FIG. 2. TBA VALUES OF RAW BUFFALO PATTIES CONTAINING ZEO AND GSE DURING STORAGE AT 8C FOR 9 DAYS.
Values with different lowercase letters are significantly different ($P < 0.05$)

of pH (Viuda-Martos *et al.* 2010). Also, samples with combination of ZEO and GSE showed higher pH values than others. Therefore, based on the pH results, it can be suggested that the combinations of ZEO and GSE had a synergistic effect on this factor (Banon *et al.* 2007).

Lipid Oxidation. Ground meat is prone to lipid damages caused by oxidative changes due to the large surface area, which might be easily reached by oxygen (Ahn *et al.* 2004). Therefore, the use of EOs and extracts with potential antioxidant power may effectively decline oxidative deterioration.

The TBA ($mg_{malondialdehyde}/kg_{sample}$) values during 9 day storage of patties containing ZEO and GSE are shown in Fig. 2. During 9 days of storage, the TBA values of treated samples were significantly lower than control ($P < 0.05$). TBA values of the control sample rapidly reached to final value of 2.01 $mg_{malondialdehyde}/kg_{sample}$, whereas samples with 0.1% ZEO reached to 0.74 $mg_{malondialdehyde}/kg_{sample}$. This amount was 14 and 20.5% lower than the TBA values of 0.1% GSE and 0.2% GSE samples (0.93 and 0.86 $mg_{malondialdehyde}/kg_{sample}$), respectively. Therefore, current results indicated higher antioxidant power of ZEO than GSE in raw buffalo patties. The results of antioxidant power evaluation in the present study were in agreement with results previously reported by Ahn *et al.* (2004) on effects of GSE (ActiVin) on prevention of lipid oxidation in raw ground meat and by Rojas and Brewer (2008) on effects of 0.02% GSE on reduction of TBA values in raw, frozen vacuum-packaged beef (Ahn *et al.* 2004; Rojas and Brewer 2008).

Dose-dependent reduction of lipid oxidation in meat products by GSE was revealed by other studies similar to the present study (Murcia and Martínez-Tomé 2001). At the end of storage time, 0.1% ZEO + 0.2% GSE samples had the lowest value of lipid oxidation (0.63 $mg_{malondialdehyde}/kg_{sample}$). The TBA value in 0.1% ZEO + 0.2% GSE samples was 68% lower than control, indicating that synergistic antioxidant activity resulted in combination of ZEO and GSE in the buffalo patties.

Antioxidant activities of ZEO could be correlated to high contents of phenolic and nonphenolic compounds (Zakipour Rahimabadi *et al.* 2013). Carvacrol and thymol are the main phenolic compounds and p-cymene is the main nonphenolic compound in ZEO (Sharififar *et al.* 2007). Considering the fact that ZEO was recognized as safe by EC as GSE, they can be practically applied as preservative in food systems (Shafiee and Javidnia 1997; Sharififar *et al.* 2007).

Sensory Evaluation. The results of sensory assessment of experimental buffalo patties are shown in Fig. 3. The control had the highest scores in all sensory attributes. Ignoring control, the scores of 0.1% ZEO were higher than other samples. Scores of color attribute in combined treated samples with ZEO and GSE were higher than other samples, but in odor and overall acceptability attributes, 0.1% ZEO + 0.2% GSE samples had the lowest scores.

Effect of ZEO and GSE on Inoculated *L. monocytogenes*

According to the results, the rate of *L. monocytogenes* growth was more rapid in control (without antimicrobial agents) and samples with GSE (either 0.1 or 0.2%)

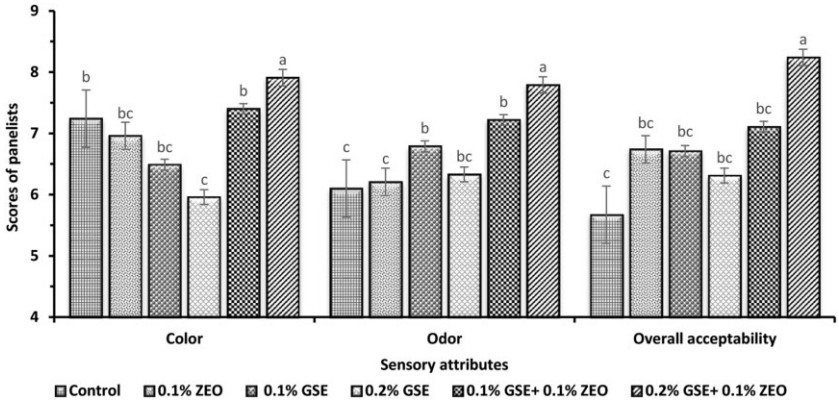

FIG. 3. SENSORY ATTRIBUTES OF RAW BUFFALO PATTIES CONTAINING ZEO AND GSE DURING STORAGE AT 8C FOR 9 DAYS

than others in buffalo patties (Fig. 4). The initial *L. monocytogenes* count of control was 5.11 $\log_{10}$ cfu/g, which increased steadily during storage and reached 7.82 $\log_{10}$ cfu/g. The final count was also 5.42 $\log_{10}$ cfu/g for 0.1% ZEO, between 6.95 and 7.26 $\log_{10}$ cfu/g in samples with GSE and between 4.64 and 5.66 $\log_{10}$ cfu/g in the combined samples with ZEO and GSE after 9 day storage time.

The results showed that the combination of 0.1% ZEO + 0.2% GSE was more efficient against *L. monocytogenes* than 0.1% ZEO alone ($P < 0.05$). According to Fu *et al.* (2007), combination of different plant-originated compounds could exert additive, synergistic or antagonistic effects depending on the type of microorganism (Fu *et al.* 2007). Our results suggested that the combinations of 0.1% ZEO and 0.2% GSE have a synergistic effect against the growth of *L. monocytogenes*.

Highly hydrophobic constituents of EOs may show limited effectiveness on foods with high fat content, because EOs will partition in the lipid fraction of food (Burt 2004). But as reported in this study, the opposite may also occur with less hydrophobic EOs, such as ZEO, which had a strong effect against *L. monocytogenes* in mediate fatty products.

Restriction of *L. monocytogenes* growth was previously reported at the concentration of 0.405% ZEO in salted fish fillets (Ekhtiarzadeh *et al.* 2012). Elizaquível *et al.* (2013) reported that 0.02% ZEO suppressed *L. monocytogenes* growth and it was in a lower concentration and short exposition time than other tested Eos (Elizaquível *et al.* 2013). Also, Ahn *et al.* (2004) reported that the numbers of *L. monocytogenes* declined by 1 log cfu/g in raw ground beef treated with 1% GSE (ActiVin, a trademark name for the GSE) after 9 days of refrigerated storage (Ahn *et al.* 2004). In the present study, susceptibility of *L. monocytogenes* against samples with GSE was similar to control during the storage. Observed incompatibility in findings of different studies, mentioned earlier, could be due to the complexity

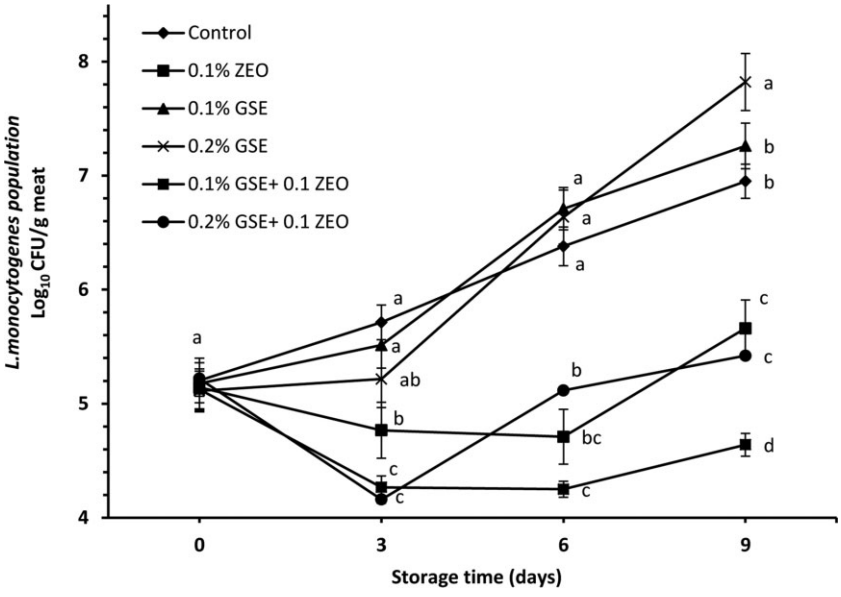

FIG. 4. EFFECT OF ZEO AND GSE ALONE AND IN COMBINATION ON LISTERIA MONOCYTOGENES COUNT IN RAW BUFFALO PATTIES DURING 9 DAYS OF STORAGE AT 8C. Values with different lowercase letters are significantly different ($P < 0.05$)

of food systems and different quantity and characteristics of the natural agents used.

CONCLUSION

The results of the present study demonstrated that combination of ZEO with different concentrations of GSE was more effective against both spoilage microorganisms and *L. monocytogenes* than using alone, indicating a synergistic antimicrobial effect in raw buffalo patty. Results also revealed that combination of ZEO and GSE had significant effects on inhibition of oxidative deterioration of buffalo meat than using alone. Hence, considering acceptable sensorial scores, additive or synergistic effects of ZEO and GSE can be practically applied in food systems especially in meat as natural antimicrobial and antioxidant agents.

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