

**Original Article****Use of *Cuminum Cyminum* Essential Oil and *Biarum carduchorum* Water Extract on Shelf-life Extension of lambs at Cold Storage**Vaez Nemati<sup>1</sup>, Morteza Khomeiri<sup>1\*</sup>, Ali Moayedi<sup>1</sup>, Alireza Sadeghi Mahoonak<sup>1</sup>, Alireza Sadeghi<sup>1</sup>, Ahad Yamchi<sup>2</sup>

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**ABSTRACT**

**Background and Objectives:** *Listeria monocytogenes* is an emerging foodborne pathogen which may be transmitted through meat and dairy products to humans. In this study, effects of *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE) and a combination of the two compounds on growth of *L. monocytogenes* and physicochemical, textural and sensory properties of lambs were investigated during cold storage.

**Materials and Methods:** Effects of CCEO and BCWE on *L. monocytogenes* were investigated after a short time of cold storage using broth microdilution assay. Furthermore, physicochemical assessments of the lamb samples were carried out, including meat quality index (pH, color, texture) and sensory analysis. Gas chromatography-mass spectroscopy (GC-MS) was used for the chemical analysis.

**Results:** The GC-MS analysis showed that  $\gamma$ -terpinene (%12.57),  $\beta$ -pinene (%11.03), geranyl acetate (%10.81), *p*-cymene (%9.95) and sabinene (%9.81) were the major components of the CCEO. Use of CCEO or BCWE alone decreased *L. monocytogenes* count to below 2 log CFU/g. Moreover, use of combined CCEO and BCWE decreased the number of *L. monocytogenes* to below the limit (2 log CFU/g) after 48 h.

**Conclusions:** Results of the physicochemical parameters of the highlighted compounds revealed their useful values since these compounds showed improvement effects with no significant changes in meat quality. However, the firmness and force necessary to cut the samples treated with CCEO decreased mildly after 72 h of storage.

**Keywords:** *Listeria monocytogenes*, *Cuminum cyminum*, *Biarum carduchorum*, Lamb, Shelf life

**Introduction**

Despite a great development in food preservation techniques, foodborne pathogens are still a continuing challenge in food safety and preservation. Of these foodborne pathogens, *Listeria monocytogenes* is a pathogenic bacterium, causing high rates of hospitalization and mortality. In fact, *L. monocytogenes* has been fully investigated in foods for the last three decades. This bacterium is known as an emerging foodborne pathogen and considered as a serious health risk due to its mortality rate, especially in newborns and the elders. Based on the reports by European Food Safety Agency, nearly 12% of the total deaths by foodborne diseases are linked to *L. monocytogenes* infections (1). As this bacterium is abundant in digestive systems of animals and in

nature, it can contaminate various foods, especially meats. Furthermore, it can easily grow and proliferate at refrigerating temperatures; therefore, storage of meats in refrigerators does not completely eliminate the risk of *L. monocytogenes* infections (2). Studies have been carried out on the effectiveness of aromatic chemical compounds extracted from plants on meat spoilage and pathogenic microorganisms in meats and meat products. Use of essential oils (EOs) to improve food safety and shelf-life of meat products has been reported mainly in fresh lamb and chicken meats (3). *Biarum carduchorum* is a member of *Araceae* family, which grows in Turkey, Syria, Iran and Iraq (4). Presence of flavonoids, anthocyanins, alkaloids, amines, saponins and cinnamic acids has been

discovered in *B. carduchorum* (5). Another herb, *Cuminum cyminum* L., is a monotypic genus in *Apiaceae* family, which grows in Northern Iran in fall and is used as meat tender. The most bioactive components of *C. cyminum* exist in its reproductive parts, especially seeds (6). The *C. cyminum* fruits are well-known appetizers used as culinary spices in food industries due to their aromatic effects (7). Studies have been carried out on antimicrobial activity of its EOs and extracts, including efficacy of *Rosmarinus officinalis* L. (rosemary) and *Thymus vulgaris* L. (thyme) EOs (8) in food models. Herbal extracts such as EOs are proven to be useful in improving color stability, preventing lipid oxidation and flavor changes in fresh meats and meat products (9). Further studies on identification of EO compounds and their antimicrobial activity are necessary to control foodborne pathogens with no undesirable effects on sensory and nutritional properties of the products. Therefore, aim of the present study was to investigate short-term (72 h) preservation and anti-listeria effects of *C. cyminum* essential oil (CCEO) and *B. carduchorum* extract (BCWE) added separately or in combination with each other to raw lambs stored at refrigerating storage conditions. For organoleptic evaluation, physicochemical parameters (pH, texture, color) were assessed in lamb samples. Moreover, sensory properties of the fresh lambs treated with CCEO and BCWE alone and a combination of both were assessed by panelists.

## Materials and Methods

### Extraction of *Cuminum cyminum* essential oil (CCEO) and gas chromatography-mass spectrometry (GC/MS) analysis

In general *C. cyminum* plants were collected in Fall of 2017 from Ramsar, Mazandaran Province, Northern Iran. Accuracy of the collected botanical information was verified by the Botanical Department, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The volatile oil of *C. cyminum* was extracted using distillation with water for 3 h and Clevenger apparatus. The extracted essential oil was stored at refrigerating temperature. The essential oil was analyzed using GC-MS (Agilent 6890, California, USA) equipped with an ion trap mass spectrometer. The GC conditions were set as follows: a HP-5MS column (30 m × 250 μm × 0.25 μ) was used; helium

gas was used for the carrier gas; column head pressure was 10.785 psi; range of oven temperature was 50–300 °C; oven temperature program isotherm included 5 min at initial temperature, then 15 °C/min to 240 °C for 3 min held at final temperature for 5 min. Identification of the components was based on their relative retention times and matching their recorded spectra with available data (10).

### Preparation and extraction of *Biarum carduchorum* water extract (BCWE)

The *B. carduchorum* was collected from Semirom in south of Isfahan Province, Iran, in April 2017 and dried in shade at 24–26 °C. Accuracy of the collected botanical information was verified by the Botanical Department of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Dry leaves were grinded and mixed using various solvents in 250 ml Erlenmeyer flasks (1:5 ratio). Water (100%) used as solvent, while the extraction process was carried out for 48 h with agitation. Extracts were filtrated using Whatman filter paper No. 1 (Merck, Germany) and rotary evaporator (RV 10 basic, IKA, USA). Filtrated extracts were dried using freeze dryer (FD-5005-BT, Dena, Iran) and stored at 4 °C until use (11).

### Bacterial strains and inoculum preparation

In this study, *L. monocytogenes* PTCC 1298 was provided by Persian Type Culture Collection (PTCC), Karaj, Iran. The bacterial strain was revived by transferring the lyophilized form to BHI (Merck, Germany) and incubating at 37 °C for 24 h. This was recultured in two consecutive experiments. The bacterial suspension turbidity was adjusted to 1 McFarland (12).

### Calculation of minimum inhibitory concentration (MIC) for *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE)

The MIC was calculated using broth microdilution susceptibility assay and serial microdilution test (13). All tests were carried out in Mueller-Hinton broth (MHB) (Quellab, Canada) supplemented with 3% of DMSO (v/v). The *L. monocytogenes* strain was cultured overnight at 37 °C in MHB. A two-fold serial dilution method was used in a range of 1000–31.25 ppm of the CCEO or BCWE in a 96-well sterile microdilution plate. First, 180 μL of the MHB containing CCEO or BCWE were added into each well of microplates. Then, 20 μL of *L. monocytogenes*

suspension containing  $3 \times 10^8$  CFU/ml were inoculated into each well of the microplate and incubated at 37 °C for 24 h. Degrees of turbidity were reported at 630 nm using microplate reader (Synergy™ HTX Multi-Mode, Biotech Instrument, USA). For the growth (MHB, DMSO and *L. monocytogenes*) and sterility (MHB, DMSO and test oils and extracts) controls, similar tests were used. When the highest dilution showed complete growth inhibition of the test bacteria, the MIC was reported (13).

#### Calculation of minimum bactericidal concentration (MBC) for *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE)

Based on the MIC results, wells representing a complete growth absence were determined and from each well, 5 µL were spread on MHA plates and incubated at 37 °C for 24 h. The complete growth inhibition was reported as the MBC (13).

#### Experimental groups

The experiment was designed to investigate short-term preserving effects of CCEO and BCWE separately and in combination using five storage time periods of 0, 12, 24, 48 and 72 h. The experiments studied inhibitory effects of the extracts using listeria-inoculated raw meats. Five technical replicates were used for each sample. The longissimus lumborum (LL) muscles of lambs were purchased five times from local butchers. The muscles were cut into  $2 \times 2 \times 2$  cm pieces aseptically, placed into sterile Petri dishes and immediately used in experiments. First, bacterial suspensions containing  $3 \times 10^8$  CFU/ml of *L. monocytogenes* were added to meat pieces. Then, lamb pieces were dipped to dilutions ranging 1000–31.25 ppm of the extract and essential oil for 30 min. These samples were stored at refrigerator and then analyzed after 0, 12, 24, 48 and 72 h of storage.

#### Antimicrobial activities of *Cuminum cyminum* essential oil (CCEO), *Biarum carduchorum* water extract (BCWE) and their combinations on lambs

Briefly, 200 g of the meat were cut into small pieces of  $2 \times 2 \times 2$  cm. Samples were sterilized using 5-Gray gamma radiation (14). Bacterial suspensions containing  $3 \times 10^8$  CFU/ml of *L. monocytogenes* were prepared and added to the meat pieces. Then, presence of *L. monocytogenes* in meat samples was investigated at various time intervals (0, 12, 24, 48, 72 h). For bacterial counting at each sampling time,

45 ml of physiological serum were mixed with raw lamb samples and homogenized using stomacher (Seward Stomacher 400C, USA) and then appropriate serial dilutions (10:1) were prepared. A 0.1-ml diluted sample was placed on *Listeria* chromogenic agar (CHROM agar, France) and incubated for 24 h at 37 °C. The *L. monocytogenes* formed colonies in blue with white halos on this media.

#### Physicochemical properties of lambs

Physicochemical properties were assessed for the control and treated lamb samples stored at refrigerator at each storage time of 0, 12, 24, 48 and 72 h.

**pH:** Briefly, pH assessment was carried out for homogenate treated and control meat samples at room temperature. The pH meter was calibrated using buffer solutions of pH 3 and pH 7 and then measurements were carried out using PHS-550 Bench-top pH Meter (Lohand, China) (15).

**Color:** Color assessment was carried out at 25 °C using LOVIBOND AF710-3 (Tintometer, Lovibond, England) and reported using CIE L\*a\*b\* system (16).

**Texture:** Texture assessment was carried out using texture analyzer (Farnell CNS, Italy). The crosshead speed was 1.5 mm/s. Treated lamb samples and control ( $2 \times 2 \times 2$  cm) were sheared once in the center and at the right angle to the longitudinal orientation of the muscles (17). Five technical replicates were used for each sample. The shear parameters were calculated from the force-deformation curves of the maximum shear peak force as firmness and the total work used to cut through the lambs.

**Sensory evaluation:** The meat sensory was evaluated by ten panelists. Lamb samples sterilized with 5-Gray gamma radiation (control, BCWE treated lamb, CCEO treated lamb and BCWC treated lamb) from each storage time were cooked in an oven at 160 °C for 20 min until reaching an internal temperature of 90 °C. Then, samples were served at plastic dishes and coded with random three-digit numbers. Samples were ranked using a 1–9 number for each parameter (color, odor, taste, texture) (18). The scale items included like extremely (9); like very much (8); like (7); like slightly (6); neither like nor dislike (5); dislike slightly (4); dislike (3); dislike very much (2); and dislike extremely (1).

#### Statistical analysis

Data were analyzed using SAS 9.1.3 Statistical Software Package for Windows. Effects of C.

*cuminum* and *B. carduchorum* were studied on anti-listeria activity, pH, color, texture and sensory evaluation of the lamb samples through a factorial design. All measurements were carried out in triplicate ( $n = 3$ ). Results were analyzed using Duncan test at a significance level of  $\alpha = 0.05$ .

## Results

### Chemical compositions of *Cuminum cyminum* essential oil (CCEO)

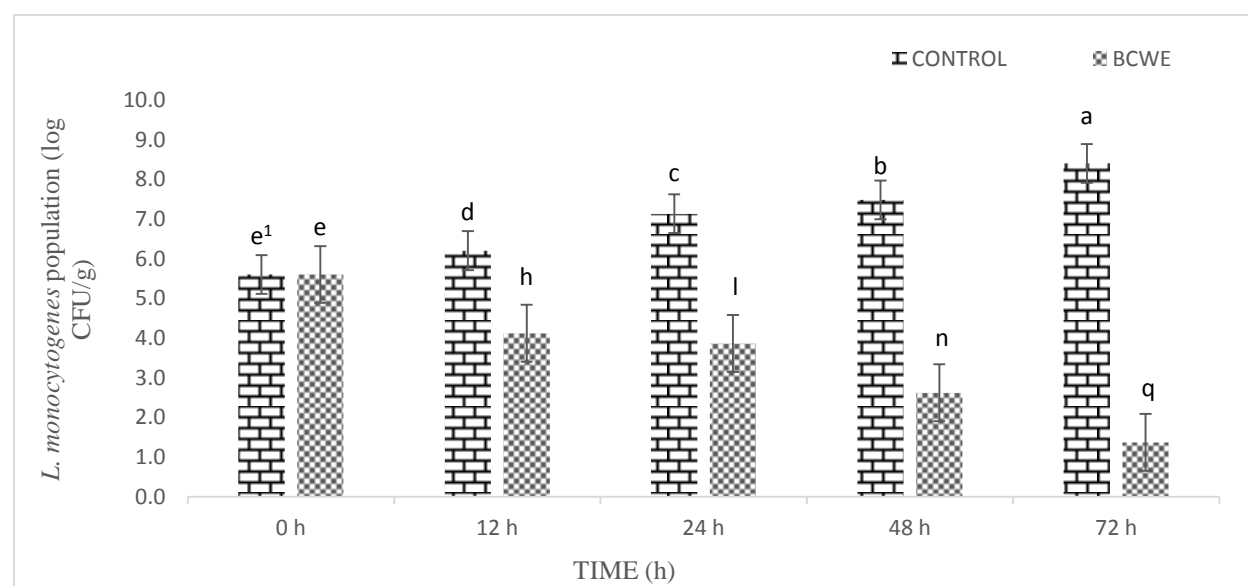
The GC-MS analysis showed  $\gamma$ -terpinene (%12.57),  $\beta$ -pinene (11.03%), geranyl acetate (10.81%), p-cymene (9.95%) and sabinene (9.81%) as the major components of CCEO (Table 1).

### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

In this study, CCEO at 62.50 ppm inhibited the growth of *L. monocytogenes* and showed bactericidal effects at 250 ppm. The BCWE included MIC at 250 ppm and BCWE with CCEO (BCWC) included 62.50 ppm. Furthermore, bactericidal effects were observed against *L. monocytogenes* at 500 ppm of BCWE and 125 ppm of BCWC.

### Effects of *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE) on lambs inoculated with *L. monocytogenes* during storage

As shown in Figure 1, the mean number of *L. monocytogenes* in lamb samples on Day 1 included 5.60 Log CFU/g. During cold storage, the number of *L. monocytogenes* in control group increased to 8.40 Log CFU/g after 72 h of storage at +4 °C. However, the number of bacteria in all extract and essential oil treatments decreased significantly, compared to that in control group ( $P < 0.05$ ). The CCEO treatment decreased the number of *L. monocytogenes* to less than 2 log CFU/g (the highest acceptable level of *L. monocytogenes* per gram of raw foods) after 24 h (Figure 1). The BCWE treatment decreased the number of *L. monocytogenes* to below 2 log CFU/g (the acceptable level of *L. monocytogenes* per gram of raw meats) after 72 h. Results showed a significant difference between the control and treated lambs with a combination of CCEO and BCWE after 12 h of storage at +4 °C ( $P < 0.05$ ) (Figure 1). Moreover, all treatments were significantly lower in bacterial load than that the control group was at the end of the experiment ( $P < 0.05$ ). However, the *L. monocytogenes* level in BCWC treatment was less than 2 log CFU/g after 48 h of storage. Synergistic effects of CCEO with BCWE in inhibition of *L. monocytogenes* are shown in Figure 1.



**Figure 1.** *Listeria monocytogenes* populations (Log CFU/g) in control and treated lambs with *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE) alone and a combination of both compounds during storage at +4°C



**Table 1.** Major chemical compositions of the *Cuminum cyminum* essential oil (CCEO)

Component	Retention index	Percent	Component	Retention index	Percent
$\gamma$ -Terpinene	9/545	12.57	Linalool	10.075	2.39
Propanal	12.035	11.03	beta-Fenchyl alcohol	11.028	2.32
Geranyl acetate	8.140	10.81	Citronellol	7.130	1.87
p-Cymene	8.231	9.95	$\beta$ -myrcene	9.630	1.75
Sabinene	9.012	9.81	Norborneol	10.951	1.72
AC1LBH3H	11.746	9.67	Methyl isovalerate	11.230	1.66
$\alpha$ -pinene	8.425	5.42	$\alpha$ -Terpinene	9.638	0.97
Terpinen-4-ol	9.630	2.97	Citral	12.090	0.73

### Physicochemical properties of the treated meats during 72 of storage

The pH values of treatments with essential oil and extract were not significantly affected in most of the cold storage time periods ( $P < 0.05$ ) (Table 2). The pH values of lamb samples ranged 5.59–5.84. The pH values of BCWE treated lambs were not significantly affected, compared to the pH values of control samples after 72 h of storage. Furthermore, BCWE treatment showed the highest decrease in pH after 72 h of storage, compared to the control sample. However, an increase in pH value could be seen in CCEO treatments over this time period, compared to the control sample. The BCWC decreased pH values after 72 h of storage, compared to the control. The pH values ranged 5.58–5.78. BCWE treatment showed the highest decrease in pH after 72 h of cold storage, compared to the control sample. Meat color values treated with CCEO and BCWE are reported in Table 3. Results showed that significant differences ( $P < 0.05$ ) were associated to  $a^*$  and  $b^*$  values between the treatments. During the storage,  $L^*$  (lightness) values of the control samples were not significantly changed, compared to treated samples. The highest  $L^*$  values were recorded in samples treated with CCEO after 24 h of storage. Redness ( $a^*$ ) of the lambs was affected by the treatments, but only during the first 24 h of the refrigerating storage. However, this variability decreased at 48 and 72 h of storage.

Treated meat samples showed lower redness values compared to control samples. At Time 0, redness values showed significant differences ( $P < 0.05$ ) in control samples. After 24 h, CCEO treated samples showed significantly lower  $a^*$  values compared to the control. After 72 h, all treated samples demonstrated an increase in  $b^*$  values of the samples treated with CCEO and BCWE. All samples showed no significant differences in  $L^*$  values throughout the storage ( $P < 0.05$ ). Therefore, storage time and treatment type could not change the lightness of the treated raw lambs. Results showed significant differences in  $a^*$  values of treated lambs with BCWC and the control during the storage. After 72 h of storage, an increase was seen in redness index of the samples treated with BCWC but  $b^*$  values of the control samples decreased. The BCWC treated samples included the lowest  $b^*$  values throughout the storage. Based on the results, it could be concluded that the treatments included no effects on color properties of the meats up to three days of refrigerating storage. As shown in Table 4, texture analysis results showed that the meat tenderness parameters of BCWE and control samples increased during the storage. Treated meat samples with CCEO mildly decreased the firmness and work of shear in lambs after 72 h of storage and treated meat samples with BCWC mildly increased during the storage.

**Table 2.** The pH values of control and treated lambs with *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE) alone and a combination of both compounds during storage at +4 °C

TIME (h)	CONTROL	BCWE	CCEO	BCWC
0h	5.79±0.03 <sup>bc</sup>	5.74±0.02 <sup>c</sup>	5.76±0.03 <sup>bc</sup>	5.78±0.03 <sup>a</sup>
12h	5.68±0.01 <sup>d2</sup>	5.63±0.03 <sup>ef</sup>	5.68±0.01 <sup>d</sup>	5.61±0.01 <sup>ghi</sup>
24h	5.65±0.02 <sup>de</sup>	5.59±0.02 <sup>f</sup>	5.66±0.01 <sup>de</sup>	5.58±0.02 <sup>i</sup>
48h	5.62±0.03 <sup>ef</sup>	5.64±0.01 <sup>de</sup>	5.72±0.04 <sup>c</sup>	5.60±0.02 <sup>hi</sup>
72h	5.66±0.01 <sup>de</sup>	5.64±0.01 <sup>de</sup>	5.75±0.02 <sup>bc</sup>	5.63±0.01 <sup>fghi</sup>

<sup>2</sup> ±SE (standard error)

Different superscript letters indicate significant differences ( $P < 0.05$ )

**Table 3.** Effects of *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE) alone and a combination of both compounds on color properties of lambs during storage at 4 °C

Sample	Storage time (h)				
	0h	12h	24h	48h	72h
<b>L*</b>					
control	55.71±0.01 <sup>h2</sup>	55.72±0.02 <sup>h</sup>	55.73±0.04 <sup>h</sup>	55.78±0.00 <sup>h</sup>	55.24±0.02 <sup>kl</sup>
BCWE	55.73±0.01	55.53±0.06 <sup>j</sup>	55.48±0.02 <sup>j</sup>	55.64±0.03 <sup>i</sup>	55.28±0.09 <sup>k</sup>
CCEO	56.30±0.02 <sup>f</sup>	55.12±0.11 <sup>m</sup>	55.78±0.01 <sup>h</sup>	55.20±0.01 <sup>l</sup>	55.79±0.02 <sup>h</sup>
BCWC	56.23±0.01 <sup>i</sup>	56.03±0.02 <sup>k</sup>	56.42±0.01 <sup>h</sup>	56.55±0.01 <sup>g</sup>	56.13±0.11 <sup>j</sup>
<b>a*</b>					
control	3.19±0.04 <sup>b</sup>	3.56±0.02 <sup>a</sup>	3.20±0.00 <sup>b</sup>	2.56±0.13 <sup>d</sup>	2.56±0.01 <sup>d</sup>
BCWE	1.83±0.03 <sup>hi</sup>	2.33±0.01 <sup>e</sup>	2.16±0.04 <sup>f</sup>	1.82±0.01 <sup>hi</sup>	2.81±0.00 <sup>c</sup>
CCEO	1.23±0.02 <sup>p</sup>	1.63±0.04 <sup>l</sup>	1.72±0.06 <sup>k</sup>	1.93±0.07 <sup>g</sup>	1.86±0.11 <sup>h</sup>
BCWC	1.83±0.02 <sup>i</sup>	2.24±0.01 <sup>f</sup>	2.09±0.01 <sup>g</sup>	1.92±0.01 <sup>h</sup>	2.73±0.02 <sup>c</sup>
<b>b*</b>					
control	9.99±0.04 <sup>a</sup>	9.71±0.03 <sup>b</sup>	9.62±0.01 <sup>c</sup>	8.92±0.05 <sup>h</sup>	8.77±0.01 <sup>i</sup>
BCWE	5.85±0.01 <sup>s</sup>	7.24±0.04 <sup>p</sup>	8.42±0.05 <sup>k</sup>	8.45±0.02 <sup>k</sup>	8.13±0.09 <sup>n</sup>
CCEO	6.22±0.05 <sup>q</sup>	7.32±0.01 <sup>o</sup>	8.32±0.01 <sup>l</sup>	8.93±0.01 <sup>h</sup>	9.35±0.01 <sup>e</sup>
BCWC	5.75±0.02 <sup>q</sup>	7.43±0.01 <sup>o</sup>	8.34±0.04 <sup>l</sup>	8.53±0.02 <sup>k</sup>	8.22±0.01 <sup>m</sup>

<sup>2</sup>±SE (standard error)Different superscript letters indicate significant differences ( $P < 0.05$ )**Table 4.** Effects of treatments with *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE) alone and a combination of both compounds on texture properties of lambs during storage at 4 °C

Sample	Storage time (h)				
	0h	12h	24h	48h	72h
<b>Texture (firmness)</b>					
control	9.7±0.01 <sup>fl</sup>	13.7±0.02 <sup>b</sup>	18.1±0.01 <sup>g</sup>	16.0±0.03 <sup>q</sup>	12.9±0.01 <sup>d</sup>
BCWE	15.2±0.02 <sup>t</sup>	16.9±0.10 <sup>l</sup>	18.2±0.02 <sup>f</sup>	18.7±0.03 <sup>d</sup>	18.3±0.01 <sup>e</sup>
CCEO	14.2±0.12 <sup>x</sup>	13.8±0.03 <sup>a</sup>	13.6±0.00 <sup>c</sup>	14.8±0.08 <sup>v</sup>	12.2±0.01 <sup>e</sup>
BCWC	14.8±0.00 <sup>v</sup>	14.1±0.02 <sup>y</sup>	13.9±0.07 <sup>z</sup>	14.2±0.04 <sup>x</sup>	15.1±0.02 <sup>u</sup>
<b>Texture (work to shear)</b>					
control	131.1±0.01 <sup>m</sup>	184. ±0.02 <sup>f</sup>	243.5±0.17 <sup>e</sup>	225.5±0.03 <sup>l</sup>	182.2±0.01 <sup>g</sup>
BCWE	204.7±0.01 <sup>x</sup>	219.5±0.03 <sup>m</sup>	253.21±0.05 <sup>d</sup>	209.8±0.01 <sup>u</sup>	211.2±0.04 <sup>t</sup>
CCEO	178.5±0.02 <sup>j</sup>	180.6±0.03 <sup>l</sup>	188.3±0.02 <sup>c</sup>	213.8±0.01 <sup>q</sup>	164.3±0.01 <sup>l</sup>
BCWC	195.8±0.01 <sup>A</sup>	212.1±0.05 <sup>s</sup>	241.8±0.03 <sup>f</sup>	229.2±0.02 <sup>j</sup>	205.8±0.02 <sup>w</sup>

<sup>1</sup>±SE (standard error)Different superscript letters indicate significant differences ( $P < 0.05$ )

### Sensory evaluation

Results of the sensory analysis are presented in Table 5. Results indicated that the treatments and storage time were significantly different ( $P < 0.05$ ). The panelists could not notice differences in meat colors recorded by instruments. Differences in treatments and storage time were significant regarding tastes of the treated lamb samples ( $P < 0.05$ ). Storage times significantly affected scores of the CCEO treated lambs since increases were seen with storage times from unacceptable to “like” score (Day 3). This was the highest score within all treated meat samples after 72 h of storage. This might be a result of the

strong scents of the meat samples, scored with the lowest grade within all samples at Time 0. As a confirmation, similar patterns of rising scores could be reported for the meat odor. Contrary to CCEO treated lambs, samples treated with BCWC demonstrated decreases in consumer taste scores, which was correlated with the time of cold storage. It is clear that all treatments included lower scores but over-limiting acceptability scores (scores lower than 5 were considered as unacceptable) than the control samples. Rare exceptions included CCEO treated lambs at Time 0 and BCWC treated lambs after three days of storage.

**Table 5.** Sensory evaluations of the lambs treated with *Cuminum cyminum* essential oil (CCEO) and *Biarum carduchorum* water extract (BCWE) alone and a combination of both compounds

Sample	Storage time (h)				
	0h	12h	24h	48h	72h
<b>Color</b>					
Control	8.14 ± 0.69	7.44 ± 0.35	7.57 ± 0.97	7.67 ± 0.51	8.00 ± 0.81
CCEO	8.00 ± 0.82	7.24 ± 0.22	7.57 ± 0.53	7.67 ± 0.51	7.86 ± 0.69
BCWE	8.00 ± 0.82	7.24 ± 0.22	7.57 ± 0.53	7.67 ± 0.51	7.86 ± 0.69
BCWC	8.00 ± 0.82	7.24 ± 0.22	7.57 ± 0.53	7.67 ± 0.52	7.86 ± 0.69
<b>Odor</b>					
Control	7.43 ± 0.79	7.05 ± 0.81	7.14 ± 0.90	7.83 ± 0.75	7.43 ± 0.97
CCEO	6.86 ± 1.21	6.32 ± 0.25	6.43 ± 1.27	6.92 ± 1.36	7.71 ± 1.25
BCWE	7.57 ± 0.98	7.11 ± 0.18	6.07 ± 1.23	6.08 ± 0.80	6.64 ± 0.94
BCWC	7.14 ± 1.21	6.55 ± 0.41	6.43 ± 1.27	6.58 ± 1.28	7.14 ± 1.21
<b>Taste</b>					
Control	7.57 ± 1.13 <sup>a</sup>	7.65 ± 1.20	7.86 ± 0.69 <sup>a</sup>	7.33 ± 0.82 <sup>a</sup>	7.14 ± 0.69 <sup>a</sup>
CCEO	4.14 ± 0.90 <sup>b,A</sup>	4.18 ± 0.68	5.79 ± 1.34 <sup>b,B</sup>	6.17 ± 0.98 <sup>a,B</sup>	6.64 ± 0.85 <sup>a,b,B</sup>
BCWE	5.86 ± 1.06 <sup>c</sup>	5.34 ± 1.12	5.14 ± 1.10 <sup>b</sup>	5.42 ± 0.66 <sup>b</sup>	5.71 ± 0.81 <sup>b,c</sup>
BCWC	5.86 ± 1.34 <sup>c,A</sup>	5.70 ± 1.24	5.57 ± 1.61 <sup>b,A,B</sup>	5.42 ± 0.92 <sup>b,A,B</sup>	4.43 ± 0.44 <sup>c,B</sup>
<b>Texture</b>					
Control	7.71 ± 0.49 <sup>A</sup>	7.52 ± 0.28	7.14 ± 0.37 <sup>B</sup>	7.33 ± 0.52 <sup>A,B</sup>	7.29 ± 0.48 <sup>A,B</sup>
CCEO	7.86 ± 0.69	7.86 ± 0.49	7.86 ± 0.37	7.67 ± 0.52	7.71 ± 0.48
BCWE	7.71 ± 0.49	7.71 ± 0.57	7.71 ± 0.48	7.83 ± 0.75	7.71 ± 0.48
BCWC	7.57 ± 0.53	7.57 ± 0.41	7.57 ± 0.53	7.50 ± 0.54	7.71 ± 0.48

Values without superscript letter are not significant

Different superscript letters indicate significant differences ( $P < 0.05$ )

## Discussion

In general,  $\gamma$ -terpinene,  $\beta$ -pinene, geranyl acetate, p-cymene and sabinene were the major components of CCEO. However,  $\beta$ -pinene, p-cymene,  $\gamma$ -terpinene, cuminal,  $\alpha$ -terpinen-7-aI, and  $\gamma$ -terpinen-7-a were the major components of CCEO in other studies (19). Furthermore, 20 species of Iranian *C. cyminum* were investigated and reported that  $\gamma$ -terpinene, cuminaldehyde, cuminalcohol and p-cymene were the major components of Iranian accessions of *C. cyminum* (20). Differences in chemical compositions of the essential oils in the current study and other studies could be occur due to variations in geographical areas of planting, planting seasons, climatic conditions, various parts of the plants, methodologies and durations of the essential oil extractions and assessments (21). In the present study, strong antimicrobial activities were seen in CCEO; also previously reported by Mir et al. (22). Complete death times for *C. cyminum* and *Rosmarinus officinalis* L. oils included 20 and 25 mins against *Escherichia coli*, 180 and 240 mins against

*Staphylococcus aureus* and 90 and 120 mins against *L. monocytogenes*. As a result of consumer demands for natural and preservative-free products, food industries pay further attentions to natural antimicrobials for the control of microbiological spoilages in food products (22). Plant extracts such as essential oils have demonstrated good antimicrobial activities against spoilage microorganisms and foodborne pathogens (23, 24). The antibacterial mechanisms of CCEO on *L. monocytogenes* were analyzed and results showed disruptive actions of essential oils on the bacterial cytoplasmic membranes. In the current study, CCEO of 750  $\mu$ L decreased number of *L. monocytogenes* from 5.60 to 3.78 Log CFU/g after seven days of cold storage (7). Similar results reported use of CCEO to inhibit *L. monocytogenes* growth in meats and extend meat shelf life. Moreover, cuminaldehyde in *C. cyminum* is reported to inhibit growth and production of bacterial toxins in meats (25). Similar studies showed effectiveness of the essential oils such as *C. cyminum* to prolong meat shelf life and inhibit bacterial growth

(26). The bright red color of lambs is traditionally considered as a positive feature since it is associated with freshness and superior quality products (25). In this study, the highest L\* values were recorded in samples treated with CCEO after 24 h of storage. These results were similar to results of a previous study by Zhang et al. (2016); in which, spice extracts were added to chicken samples, which constantly increased L\* values during a storage period of 15 days (26). Texture analysis results showed that meat tenderness parameters of BCWE and controls increased during the storage time. Treated meat samples with CCEO mildly decreased the firmness and work of shear in lambs after 72 h of storage, and treated meat samples with BCWC mildly increased during the storage time. Sarcomere length and shear force values are commonly used for measuring this quality parameter. A relationship between sarcomere length and meat toughness has been described with short sarcomeres resulting in less tender meats (19). Shear force values are usually used to measure meat toughness; thus, high shear force values were significantly linked to tough meats (20). Based on the sensory analysis, the panelists could not report differences in meat colors that were recorded by instruments. Similar results were reported by Fernandes et al. (2016) in sheep burgers; in which, addition of oregano extracts prevented the loss of sensory quality up to 15 days of storage. Sensory analysis of lamb meats additionally demonstrated that at the higher concentrations of thyme and oregano essential oils resulted in stronger odors and tastes (9).

### Conclusion

The present study showed potential effects of *Cuminum cyminum* essential oil and *Biarum carduchorum* water extract alone and their combinations on shelf-life extensions of lambs during refrigerating storages. The *Cuminum cyminum* essential oil and *B. carduchorum* water extract were able to decrease *Listeria monocytogenes* viable counts in lambs. Further results of physicochemical parameters revealed usefulness of these compounds since they demonstrated beneficial effects on pH with no undesirable changes in texture and color of meats. Furthermore, treated meat samples with *Cuminum cyminum* essential oil improved textures of the meats.

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