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Effects of water extract of *Urtica dioica* L. and modified atmosphere packaging on the shelf life of ground beef

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ABSTRACT

Effects of lyophilized *Urtica dioica* L. water extract (LUWE) and modified atmosphere packaging (MAP) on the quality and shelf life of ground beef were investigated. Ground beef was stored as aerobic control, MAP (80%O_2 + 20% CO_2), MAP + 250 ppm LUWE and MAP and 500 ppm LUWE at 2 ± 0.5 °C for 14 days. MAP and LUWE had significant effects on mesophilic, psychrotrophic and lactic acid bacteria and *Pseudomonas* counts. Depending on the level of LUWE, *Pseudomonas* and psychrotrophic counts decreased. Treatment with 500 ppm LUWE + MAP showed the lowest TBARS values compared to other groups during storage. 80% O_2-MAP increased TBARS values. Treatment had no significant effect on L^* and b^* values of the exterior of the ground beef, but had significant effects on the color of interior sections.

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1. Introduction

Plant extracts with antioxidant and antimicrobial activities are being studied to prolong the shelf life of meat and meat products. Dried-granulated *Urtica dioica L.* leaves have important antioxidant (Aksu, 2003) and antimicrobial (Aksu & Kaya, 2004) effects on fermented meat products. A *U. dioica L.* water extract had more antioxidant activity than BHA, quercetin, and α-tocoferrol, and also had antimicrobial effects on various bacteria in *vitro* (Gülçin, Küfrevioğlu, Oktay, & Büyükokuroğlu, 2004).

Ground beef is more susceptible to lipolytic reactions because of its larger surface area compared to other meat products (McMillin, 2008). One way to prevent lipid oxidation is by using natural and/or synthetic antioxidants in meat products (Morrissey, Sheey, Galvin, Kerry, & Buckley, 1998; Tang et al., 2006). Some of the synthetic chemicals used to prevent lipid oxidation may be detrimental to health (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2003, Buxiang & Fukuhara, 1997; Kansci, Genot, Meynier, & Grandemer, 1997). Therefore, natural antioxidants should replace synthetic antioxidants in the medical and food sectors (Kumaran & Karunakaran, 2007). Some plants such as *U. dioica* L. can be a good source of antioxidants. A *U. dioica* L. extract prevented free radical formation in lipid oxidation (Exarchou, Fiamegos, Vanbeek, Nanos, & Vervoort, 2006) and *U. dioica* L. leaves are a good source of essential amino acids, ascorbic acid and other minerals (especially Fe) (Martinez, Djenane, Cilla, Beltran, & Roncales, 2006). *U. dioica* L. possesses antioxidant activity (Aksu, 2003; Gülçin et al., 2004; Karabacak & Bozkurt, 2008) but, there are no reports on the antioxidant activity of *U. dioica* in fresh meats.

U. diocia L. is heavily used in the medical sector due to its antiviral properties. *U. dioica* L's and some other plants' inhibition on HIV were investigated and it was claimed that *U. dioica* L. had a strong inhibitory effect on infected cells (Manganelli, Zaccaro, & Tomei, 2005).

Shelf life of products can be prolonged using MAP, generally, mixtures of CO_2, O_2 and N_2 (Philips, 1996). It was reported that the most suitable mixture for fresh meat and meat products is 80% O_2 + 20% CO_2 (Manu-Tawiah, Amman, Sebranek, & Molins, 1991).

Ground beef is an easily spoiled meat product. Microorganisms on the surface of the meat spread and multiply under suitable conditions, especially during mincing and mixing leading to a shorter shelf life and potential risks for human health. Therefore, by adding antimicrobials and antioxidants to ground beef and packaging in modified atmosphere, it is possible to extend its shelf life. No research that has been conducted into the effect of LUWE addition on the chemical and microbiological quality of ground beef during storage. The present study was conducted to determine the effect of LUWE (250 ppm and 500 ppm) and MAP (80% O_2 + 20% CO_2) during storage (0, 3, 6, 8, 11, and 14 days) on the mesophilic, psychrotrophic and lactic acid bacteria, *Pseudomonas*, *Enterobacteriaceae* counts, TBARS and color (L^*, a^* and b^*) of ground beef at 2 ± 0.5 °C.

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2. Materials and methods

2.1. Materials

Beef, from carcasses (EBK Slaughterhouse, Erzurum) was ground through a 3 mm diameter mincer under hygienic conditions, then transported, under refrigeration to the laboratory, where it was kept at 4 °C until used (approximately an hour). For the *U. dioica* L. extract, young leaves were collected from Rizekent (Erzurum/Aziziye). For packaging, PA/PE (Polyamide/Polyethylene, 15×25 cm) (3-seal bags GB 70) (O_2 permeability 40 cm^3/m^2/day.atm. 23 °C; N_2 permeability 24 cm^3/m^2/day.atm. 23 °C; CO_2 permeability 1454 cm^3/m^2/day.atm. 23 °C; and water vapor permeability <3 g/m^2/day.atm. 23 °C) was used.

2.2. Preparation of the LUWE and determination of optimal level in ground beef

U. dioica L. leaves were dried (3 days at 30–35 °C, maximum moisture level: 1–2%), then ground and sifted through a 14 inch sieve (Aksu, 2003). 20 g of the material in the bottom of the sieve was added to 400 ml of boiling water and mixed by magnetic stirrer for 15 min (Gülçin et al., 2004). After filtering (Whatman No.1) it was frozen at −38 °C and lyophilized at −50 °C.

To determine the suitable level of addition of the *U. dioica* L. water extract different amounts of extract were added to 200 g samples of ground beef and some physical and sensory properties (color, pH, cooked quality, and sensory analysis) determined. It was found that the maximum level of lyophilized *U. dioica* L. extract was 500 ppm/kg. Thus, two levels of lyophilized *U. dioica* L. water extract (250 ppm and 500 ppm) were studied.

2.3. Preparation the ground beef samples, addition of the LUWE and packaging

12 kg of ground beef was used for each experiment; which was divided into four groups for each treatment. The first group of samples (15 lots of 200-g) were put into the packaging material, then stored as the aerobic control. The second group was packaged in modified atmosphere (80% O_2 + 20% CO_2) as the MAP control To the third group of samples 250 ppm lyophilized *U. diocia* L. water extract was added and then packaged in modified atmosphere (80% O_2 + 20% CO_2). The fourth group was prepared with 500 ppm lyophilized *U. diocia* L. water extract and packaged in modified atmosphere (80% O_2 + 20% CO_2). The lyophilized *U. diocia* L. water extract was mixed with the meat by hand (with gloves). For modified atmosphere packaging, a Multivac gas packing unit (Multivac 300/16 Sepp. Haggenmuller D 87787 Wolgertschwenden, Germany) was employed.

2.4. Storage of the samples

All samples were stored at 2 ± 0.5 °C for up to 14 days. Total aerobic mesophylic bacteria, psychrotrophic bacteria, *Pseudomonas*, Lactic acid bacteria and *Enterobacteriacea* counts, thiobarbituric acid reactive substance (TBARS) and color values (L^*, a^* and b^*) were determined on the 0th, 3 rd, 6th, 8th, 11th and 14th days of the storage.

2.5. Microbiological analysis

25 g of ground beef was aseptically taken from the samples and placed in stomacher bags, with 225 ml of saline solution (NaCl 0.85%) and homogenized for 1 min. Serial dilutions of the homogenates were prepared and the surface plate method was used for enumeration. For total aerobic mesophilic bacteria (TAMB), Plate Count Agar (PCA, Oxoid) was used and plates were aerobically incubated at 30 °C for 48 h. Psychrotrophic bacteria were enumerated on PCA after incubation at 10 °C for 7 days. *Pseudomonas* was plated on Cetrimid–Fusidin–Cephaloridin (CFC) Selective Agar Supplement (Oxoid SR 0103) supplemented CFC Agar (*Pseudomonas* Agar–Base–Oxoid CM 0559) and incubated at 25 °C for 48 h. For lactic acid bacteria, MRS Agar (de Man Rogosa Sharpe Agar) was used and plates were anaerobically incubated at 30 °C for 48 h. VRBD Agar (violet red bile dextrose, oxoid) was used for *Enterobacteriacea* and plates were anaerobically incubated at 30 °C for 48 h (Baumgart, Firnhaber, & Spcher, 1993).

2.6. Determination of TBARS and color values

For TBARS values, 1 g of ground beef was added to 6 ml TCA solution (7.5% TCA, 0.1% EDTA, 0.1% 1-propyl gallate, 1-propyl gallate in 3 mL ethanol). The mixture was filtered (Whatman 1) after homogenization for 15–30 s. Then, 1 ml filtrate was added to 1 ml of 0.02 M thiobarbituric acid solution. This mixture was kept in a boiling water bath for 40 min then cooled, centrifuged at 2000×*g* for 5 min and the absorbance measured at 532 nm (Shimadzu, UV 160). TBARS values were determined as µmol malonaldehyde/kg (Lemon, 1975).

A Minolta model Colorimeter (CR-200, Minolta Co, Osaka, Japan) was used for the color measurement on both internal and external surfaces of the samples. For color readings of internal surfaces, the meat was sliced and the cut (interior) surface measured. The instrument was calibrated with standard plates before analysis. [$L^*=0$, darkness; $L^*=100$, lightness (darkness/lightness); a^*; $+a^*$ = red, $-a^*$ = green and b^*; $+b^*$ = yellow, $-b^*$ = blue].

2.7. Statistical analysis

A completely random block design was used. Results were analyzed by SPSS (1996). Comparisons of mean values were made using Duncan's multiple range test (significance $p<0.05$). The model included treatment (aerobic control, MAP control, 250 ppm LUWE + MAP and 500 ppm LUWE + MAP) and storage period (0th, 3rd, 6th, 8th, 11th, and 14th days) as the main factors, and all of their interactions. The results of the statistical analyses are shown as means ± standard deviations.

3. Results and discussion

3.1. Microbiological changes

Treatment had a significant ($p<0.01$) on TAMB counts. The highest average TAMB count was in the aerobic control while the lowest counts were in the MAP control and 250 ppm LUWE added samples. Storage time had very significant ($p<0.01$) effects on TAMB counts increasing during storage ($p<0.01$) (Table 1). Also, treatment/storage time interaction was very significant ($p<0.01$). TAMB counts did not differ significantly between the 0th and 6th days of storage. However, TAMB counts increased rapidly in aerobically packaged samples after the 6th day (Fig. 1a). In the 80% O_2 + 20% CO_2 MAP packages, CO_2 was probably the most effective antimicrobial factor. This is in agreement with the results of many studies such as Değirmencioğlu, Esmer, İrkin, and Değirmencioğlu (2008) who reported that total aerobic mesophilic bacterial counts can be decreased by packaging ground beef in a modified atmosphere (70% O_2 + 30% CO_2) and the total aerobic mesophilic bacteria in fresh meat packaged with three different modified atmosphere combinations (40% CO_2 + 30% N_2 + 30% O_2, 100% CO_2 and 80% CO_2 + 20% O_2, atmospheric combination) and vacuum packaged when stored at 0, 5, 10, 15 °C were lower than in samples stored aerobically at the same temperature (Skandamis & Nychas, 2002). Similarly, Jakobsen and Bertelsen (2004) reported that a wide group of microorganisms can be inhibited by MAP. However, *U. dioica* L. may also inhibit mesophilic bacteria.

Table 1
Pooled means for the main effects of treatment and storage time on TAMB, Psychrotrophic bacteria, *Pseudomonas*, lactic acid bacterial counts (log cfu/g) and TBARS values of control, MAP and MAP + LUWE samples.

	Total aerobic mesophilic bacteria[1]	Psychrotrophic bacteria[1]	*Pseudomonas*[1]	Lactic acid bacteria[1]	TBARS[2]
Treatment (T)					
Aerobic control	4.98 ± 1.64 a	4.99 ± 1.63 a	4.63 ± 2.04 a	3.43 ± 1.00 b	46.05 ± 33.13 b
MAP[3] control	3.94 ± 1.07 c	4.08 ± 1.15 b	2.88 ± 0.62 b	3.19 ± 1.15 c	56.20 ± 40.45 a
250 ppm LUWE[4] + MAP	3.90 ± 0.91 c	4.12 ± 1.21 b	2.68 ± 0.43 c	3.40 ± 0.89 b	40.62 ± 31.95 c
500 ppm LUWE[4] + MAP	4.15 ± 0.99 b	3.87 ± 0.922 c	2.80 ± 0.67 bc	3.59 ± 1.23 a	18.22 ± 7.98 d
Significance	**	**	**	*	**
Storage time (ST, days)					
0	3.30 ± 0.50 e	3.25 ± 0.50 e	2.34 ± 0.28 f	2.62 ± 0.53 f	7.97 ± 4.01
3	3.44 ± 0.65 e	3.39 ± 0.58 de	2.54 ± 0.54 e	2.78 ± 0.52 e	21.86 ± 8.76
6	3.61 ± 0.63 d	3.52 ± 0.49 d	2.72 ± 0.61 d	3.16 ± 0.75 d	28.59 ± 12.40
8	4.22 ± 0.100 c	4.09 ± 0.84 c	3.19 ± 0.88 c	3.60 ± 1.00 c	48.90 ± 22.34
11	4.96 ± 0.80 b	5.35 ± 0.98 b	3.88 ± 1.39 b	3.88 ± 1.05 b	59.08 ± 28.40
14	5.92 ± 1.23 a	6.00 ± 1.15 a	4.81 ± 1.98 a	4.37 ± 1.27 a	75.26 ± 46.48
Significance	**	**	**	**	**
T × ST	**	**	**	**	**

[1]log cfu/g, [2]μmol malonaldehyde/kg (Note: 100 μmol malonaldehyde/kg meat is equivalent to 7.2 ppm malonaldehyde), [3]80% O_2 + 20% CO_2, [4]LUWE: Lyophilized *Urtica dioica* L. Water Extract, $^{*}p<0.05$, $^{**}p<0.01$.
a–e, Means in the same column and in the same section having the same letters are not significantly different at $p>0.05$.

Treatment had a very significant effect ($p<0.01$) on psychrotrophic bacterial counts. The highest average psychrotrophic counts were in the aerobic control, and the lowest in the 500 ppm LUWE added samples. Storage time had very significant effects ($p<0.01$) on psychrotrophic bacterial counts (Table 1). Also treatment × storage time had a very significant effect ($p<0.01$) on psychrotrophic counts (Fig. 1b). Psychrotrophic bacterial counts increased during storage in the aerobic control and were significantly higher after 11 and 14 days, compared to the other treatments, there were no differences in psychrotrophic counts among treatments up to day 8 of storage (Fig. 1b). *U. dioica* L. had a small but significant effect on inhibiting psychrotrophic bacteria.

Decreasing temperatures may favor *Pseudomonas* bacteria during the storage of fresh meats. However, in this study, it was seen that by adding LUWE to the ground beef samples, *Pseudomonas* can be inhibited. Treatment had very significant effects ($p<0.01$) on *Pseudomonas* bacterial counts. The highest average *Pseudomonas* counts were in the aerobic control and the lowest in the 250 ppm lyophilized *U. dioica* L. added samples (Table 1). Storage time and treatment × storage time interaction had very significant effects ($p<0.01$) on *Pseudomonas* counts. MAP control and LUWE added samples had lower mean counts than the aerobic group during storage (Fig. 1c). Gülçin et al. (2004) found that different microorganisms including *Pseudomonas aeruginosa* can be inhibited by *U. dioica* L.

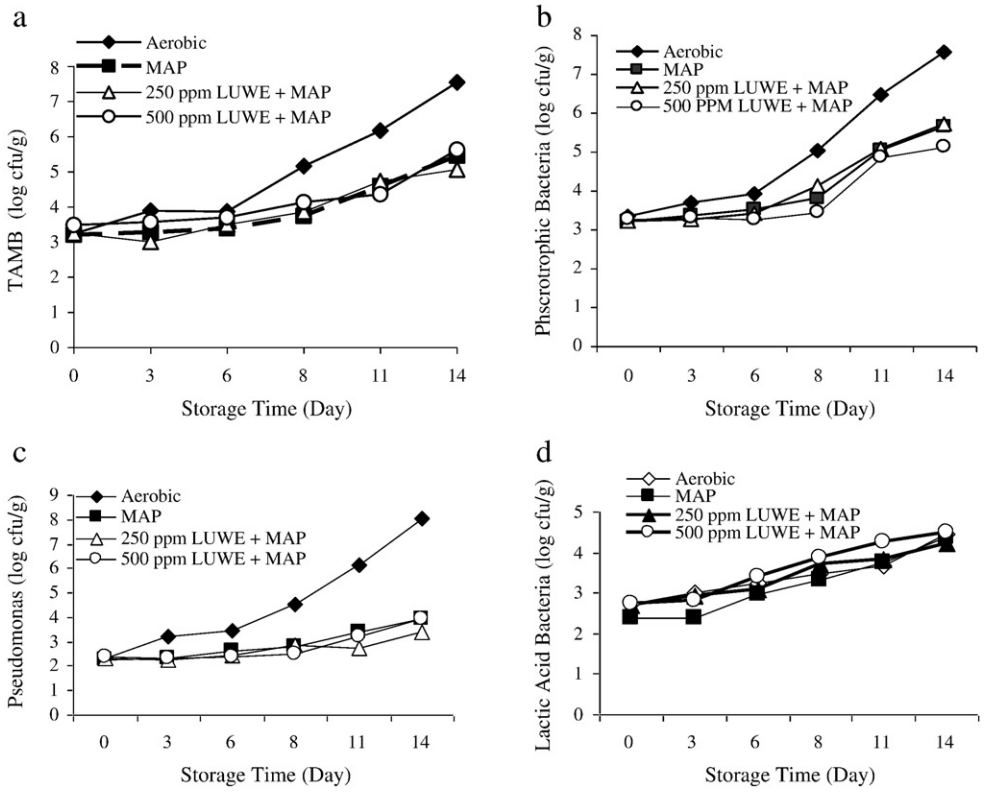

Fig. 1. Interaction effects of treatment and storage time on Total Aerobic Mesophilic Bacteria (TAMB) (a), Psychrotrophic (b), *Pseudomonas* (c) and lactic acid bacteria (d) counts of control, MAP, MAP + 250 ppm and 500 ppm LUWE at 2 ± 0.5 °C.

The highest lactic acid bacterial counts were in the 500 ppm LUWE added samples and the lowest in the MAP control (Table 1). Storage time had very significant effects ($p<0.01$). Lactic acid counts increased during storage in the 500 ppm LUWE added samples (Fig. 1d). The MAP control was stable to the 3rd day; then it increased (Fig. 1d). Treatment × storage time main effects were both highly significant ($p<0.01$) on lactic acid bacterial counts (Table 1). Lactic acid bacteria, facultative anaerobes, can grow in high concentrations of CO_2, and can be the dominant flora in modified atmosphere packed meats (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007).

As shown in Table 2, *Enterobacteriacea* counts in the 80% O_2 + 20% CO_2, MAP controls and the 80% O_2 + 20% CO_2 + 250 ppm LUWE samples were below the detection level until day 8 of storage for most samples in replication 1, and for all samples in replication 2. Similarly, Gülçin et al. (2004) and Aksu and Kaya (2004) reported that an *U. dioica* L. extract inhibited *Enterobacteriacea*.

3.2. TBARS values

The highest TBARS values were in MAP controls because of their high concentrations of O_2 (Table 1). This is in agreement with Jayasingh, Cornforth, Carpenter, and Whittier (2001) and McMillin (2008) who reported higher O_2 levels caused lower color and flavor acceptability and increased lipid oxidation during storage. Vissa and Cornforth (2006) found that TBARS values of fresh meat samples in MAP of 80% O_2 stored at 2 °C for 14 days were higher than in samples with added antioxidants. Kennedy, Buckley, and Kerry (2004) also reported that TBARS values of the MAP (80% O_2 + 20% CO_2) lamb meats stored at 2 °C for 12 days was higher than in samples stored in other gas compositions. Ground beef samples packed in modified atmosphere (70% O_2 + 30%CO_2) and stored at 4 °C for 7 days had higher TBARS values than those vacuum packed and packed in low O_2 level MAP (10% O_2 + 30% CO_2 + 60% N_2) (Değirmencioğlu et al., 2008). In the present study the lowest values were in the 500 ppm LUWE added samples (Table 1). Lipid oxidation increased during storage in all samples ($p<0.01$), however the rate of the increase in the MAP control was higher than in the other samples while the 500 ppm LUWE added samples had the lowest rate of increase (Fig. 2). *U. dioica* L. water extract has antioxidant activity dependent on the level of addition (Gülçin et al., 2004) and while *U. dioica*, nitrite/nitrate, BHT and *Hibiscus sabdariffa* all decrease TBARS values, *U. dioica* is more effective than nitrite/nitrate and BHT (Karabacak & Bozkurt, 2008).

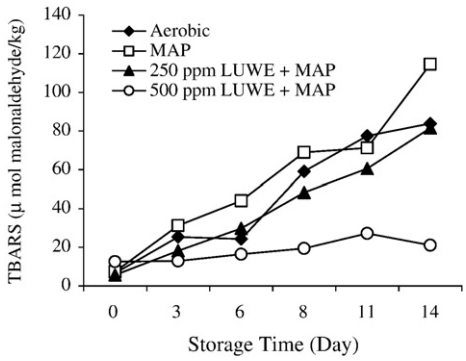

Fig. 2. Interaction effects of treatment and storage time on TBARS values of control, MAP, MAP + 250 ppm and 500 ppm LUWE at 2 ± 0.5 °C (Note: 100 µmol malonaldehyde/kg meat is equivalent to 7.2 ppm malonaldehyde).

3.3. Color changes

3.3.1. External surface

As shown in Table 3, treatment had no significant effect ($p>0.05$) on L^* of the external surface of the samples, but storage time and interaction of treatment × storage time had very significant effects ($p<0.01$) on L^* (Fig. 3a). During storage, L^* ranged from 39.37 to 41.91 (Table 3). During storage, there was no stability among the groups (Fig. 3a). Karabacak and Bozkurt (2008) found that using low concentrations of *U. dioica* extracts had no effect on L^* values of fermented meat. On the other hand, ground beef samples packaged with a 70% O_2 + 30% CO_2 and stored at 4 °C for 7 days had better color values than controls (Değirmencioğlu et al., 2008).

The significant effect ($p<0.01$) of treatment on a^* of the external surface can be seen in Table 3, the highest value (23.87) related to the 80% O_2 MAP control. Storage time had a very significant effect ($p<0.01$) on a^*. As seen in Table 3, a^* decreased during storage. After day 11 of storage, the aerobic control value increased, whereas in the other groups it continued to decrease (Fig. 3b). Kennedy et al. (2004) observed that modified atmosphere packed (80% O_2 + 20% CO_2) lamb meat stored at 4 °C for 12 days had higher a^* values between the 9th–12th days of the storage than other MAP compositions had. In agreement, treatment × storage time interactions had very significant effects on a^* values of the external surface (Fig. 3b).

Treatment had no significant effect ($p>0.05$) while storage time had a very significant effect on b^* values of the external surface

Table 2
Enterobacteriacea counts of control, MAP and MAP + LUWE samples (log cfu/g).

Treatment	Block	Storage time (day)					
		0	3	6	8	11	14
Aerobic control	1	2.00	2.69	2.60	2.00	2.47	2.30
		2.30	2.77	2.69	2.77	2.77	2.77
	2	<2.00	<2.00	2.77	2.95	3.00	3.00
		<2.00	<2.00	2.30	3.04	2.77	3.30
MAP[1] control	1	<2.00	<2.00	2.30	<2.00	2.00	2.30
		<2.00	<2.00	2.00	<2.00	2.60	2.60
	2	<2.00	<2.00	<2.00	2.00	2.60	3.17
		<2.00	<2.00	<2.00	2.30	2.77	3.00
250 ppm LUWE[2] + MAP	1	2.00	<2.00	2.47	2.30	2.30	2.00
		2.30	<2.00	2.30	2.30	2.60	2.77
	2	<2.00	<2.00	<2.00	<2.00	2.60	3.07
		<2.00	<2.00	<2.00	<2.00	2.00	3.60
500 ppm LUWE[2] + MAP	1	<2.00	<2.00	<2.00	<2.00	2.00	2.00
		<2.00	<2.00	<2.00	<2.00	2.30	2.30
	2	<2.00	<2.00	<2.00	2.47	2.30	3.17
		<2.00	<2.00	<2.00	2.30	2.00	2.60

[1]80% O_2 + 20% CO_2, [2]LUWE: Lyophilized *Urtica dioica L.* Water Extract.

Table 3
Pooled means for the main effects of treatment and storage time on external surface L^*, a^*, b^* values of control, MAP, MAP + 250 ppm and 500 ppm LUWE samples during storage.

	L^*	a^*	b^*
Treatment (T)			
Aerobic control	41.13 ± 3.69	19.81 ± 4.78 c	10.04 ± 1.54
MAP[1] control	40.23 ± 2.91	23.87 ± 4.67 a	10.48 ± 1.87
250 ppm LUWE[2] + MAP	40.34 ± 3.76	21.07 ± 5.69 b	10.41 ± 1.49
500 ppm LUWE[2] + MAP	40.95 ± 3.66	20.04 ± 4.95 bc	10.27 ± 1.69
Significance	NS	**	NS
Storage time (ST, days)			
0	39.37 ± 2.54 c	26.39 ± 3.70 a	10.80 ± 2.05 a
3	41.28 ± 3.62 ab	23.91 ± 3.57 b	11.15 ± 1.32 a
6	40.16 ± 3.93 bc	21.50 ± 3.72 c	9.40 ± 1.54 d
8	41.91 ± 3.92 a	20.33 ± 3.34 c	10.15 ± 1.14 bc
11	41.42 ± 2.73 ab	18.84 ± 6.38 d	10.67 ± 1.84 ab
14	39.82 ± 3.63 bc	16.21 ± 3.17 e	9.65 ± 1.16 cd
Significance	**	**	**
T × ST	**	**	**

[1]80% O_2 + 20% CO_2, [2]LUWE: Lyophilized *Urtica dioica L.* Water Extract.
NS: Not significance, **$p<0.01$.

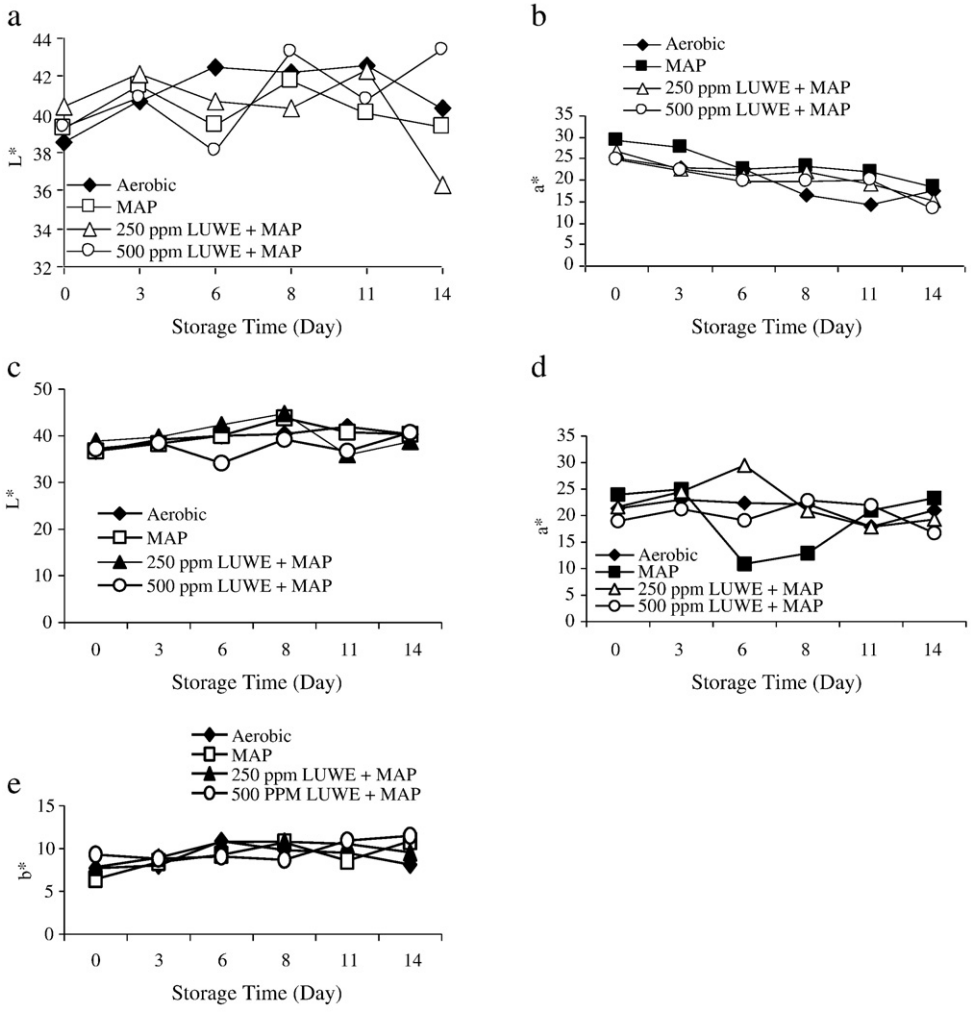

Fig. 3. Interaction effects of treatment and storage time on L^* (a) and a^* (b) values of external surface, L^* (c), a^* (d), and b^* (e) values of internal surface of control, MAP, MAP + 250 ppm and 500 ppm LUWE at 2 ± 0.5 °C.

(Table 3). Interaction of treatment × storage time also had very significant effects ($p<0.01$). In this study, values of b^* were not stable during storage (Table 3), however, Seydim, Acton, Hall, and Dawson (2006) determined that aerobic packaged ostrich meat and MAP ones in high O_2 had decreasing b^* values during storage.

3.3.2. Internal surface

Significant effects ($p<0.05$) of treatment on L^* can be seen in Table 4. The lowest values (37.72) were in the 500 ppm LUWE added samples. Storage time had a very significant effect ($p<0.01$) on L^*. Values of L^* increased until the 8th day (Table 4). All groups had similar trends in L^* during storage (Fig 3c). Treatment × storage time interactions had a significant effect ($p<0.05$) on L^* of the internal surface. Table 4 shows the significant effect of treatment ($p<0.01$) on a^* of the internal surface. The highest values were determined in the 250 ppm LUWE added samples. Similarly, Andrea, Stetzer, Novakofski, & Brewer (2008) reported that natural antioxidants, specifically rosemary extract, increased a^* (redness) and chroma (color intensity) of beef samples.

Storage time had a significant effect ($p<0.01$) on a^*, but there was no stability among the groups (Table 4). As seen in Figure 3d, the change was not stable in all groups. Also treatment × storage time had a very significant effect ($p<0.01$) on a^* of the internal surface of the samples. Both treatment and storage time had small, but very significant ($p<0.01$) effects on b^* of the internal surface (Table 4). b^* values of the 500 ppm LUWE added samples increased from the 8th day of the storage while in the aerobic controls they decreased (Fig. 3e). Treatment × storage time interaction also had very significant effects ($p<0.01$) on b^*.

Table 4
Pooled means for the main effects of treatment and storage time on internal surface L^*, a^*, b^* values of control, MAP, MAP + 250 ppm and 500 ppm LUWE samples during storage.

	L^*	a^*	b^*
Treatment (T)			
Aerobic control	39.74 ± 5.05 a	21.29 ± 4.25 ab	8.99 ± 2.10 b
MAP[1] control	40.00 ± 5.31 a	19.47 ± 6.32 c	9.02 ± 2.38 b
250 ppm LUWE[2] + MAP	40.08 ± 4.24 a	22.26 ± 6.65 a	9.72 ± 1.64 a
500 ppm LUWE[2] + MAP	37.72 ± 3.65 b	20.12 ± 5.43 bc	9.70 ± 1.52 a
Significance	*	**	*
Storage time (ST, days)			
0	37.38 ± 3.18 c	21.47 ± 3.13 b	7.80 ± 1.66 b
3	38.92 ± 62.37 bc	23.41 ± 2.94 a	8.53 ± 1.20 b
6	39.13 ± 6.39 bc	20.44 ± 8.19 bc	9.97 ± 2.18 a
8	42.03 ± 4.27 a	19.68 ± 6.51 c	9.98 ± 1.58 a
11	38.81 ± 5.39 bc	19.65 ± 5.42 c	9.87 ± 1.42 a
14	40.03 ± 4.34 ab	20.05 ± 6.39 bc	9.99 ± 2.36 a
Significance	**	**	**
T × ST	*	**	**

[1]80% O_2 + 20% CO_2, [2]LUWE: Lyophilized *Urtica dioica L.* Water Extract.
*$p<0.05$, **$p<0.01$.

4. Conclusion

Inhibition of *Pseudomonas* and psychrotrophic bacteria in the ground beef stored at 2 ± 0.5 °C for 14 days is achieved using a 500 ppm LUWE + MAP combination. Lipid oxidation can be inhibited by LUWE. The quality of ground beef can be protected best using 500 ppm LUWE and MAP with 80% O_2 + 20% CO_2. Using only MAP with 80% O_2 + 20% CO_2 is unsuitable for ground beef because lipid oxidation increases. Thus, for extended refrigerated storage of ground beef in 80% O_2 + 20% CO_2, antioxidant addition is recommended.

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