EFFECT OF LYOPHILIZED WATER EXTRACT OF *URTICA DIOICA* L. ON THE SHELF LIFE OF VACUUM-PACKAGED BEEF STEAKS

MUHAMMET IRFAN AKSU[1], HAMIDEH ALINEZHAD and EBRU ERDEMIR

Faculty of Agriculture, Department of Food Engineering, Ataturk University, Erzurum 25240, Turkey

[1]Corresponding author.
TEL: +90-442-231-27-24;
FAX: +90-442-236-09-58;
EMAIL: miaksu@atauni.edu.tr;
miaksu@hotmail.com

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ABSTRACT

In this research, the effects of lyophilized *Urtica dioica* L. water extract (LUDWE) on the shelf life of vacuum-packaged beef steaks were evaluated. For this purpose, beef steaks were arranged as control (vacuum packaging, VP), 150 ppm LUDWE + VP, 300 ppm LUDWE + VP and 450 ppm LUDWE + VP, and were stored at 2 ± 0.5C for 21 days. During storage, psychrotrophic bacteria, lactic acid bacteria, *Pseudomonas*, Enterobacteriaceae counts, pH, color and thiobarbituric acid reactive substances (TBARS) values of beef steaks were determined. By adding LUDWE in the beef steaks, TBARS values decreased ($P < 0.01$), and minimum values were determined in 450 ppm LUDWE + VP groups ($P < 0.05$). Depending on the increase in the LUDWE level, psychrotrophic ($P < 0.01$), *Pseudomonas* ($P < 0.01$), Enterobacteriaceae ($P < 0.01$) and lactic acid bacteria ($P < 0.01$) counts decreased, and the highest counts of these parameters were determined in the control group. LUDWE also had significant effects on L^* ($P < 0.01$), a^* ($P < 0.01$) and b^* ($P < 0.01$) values of vacuum-packaged beef steaks.

PRACTICAL APPLICATIONS

Beef steak is more susceptible to microbial spoilage and lipolytic reactions; these can lead to a shorter shelf life with potential risks for human health during storage at low temperatures. In beef steaks, lipid oxidation can be decreased and spoilage bacterial counts can be suppressed using natural antioxidants and antimicrobials such as lyophilized *Urtica dioica* L. water extract (LUDWE). This work clearly showed that LUDWE could be used as a good natural antioxidant and antimicrobial agent in fresh meat products such as beef steaks.

INTRODUCTION

The shelf life of beef meat can be defined as the time period between packing and until the consumer acquires the beef meat, including acceptable nutritive value, flavor, color, appearance and texture (Singh and Singh 2005). In fresh beef meats, lipid oxidation or rancidity is one of the main causes of spoilage during storage. Effective natural and/or synthetic antioxidants are added to meats in order to retard or reduce the lipid oxidation (Morrissey *et al.* 1998; Tang *et al.* 2006; Alp and Aksu 2010; Monfared *et al.* 2011). In fresh meat and meat products, synthetic antioxidants have been used for a long time, but in recent years, they have come into dispute because of their detrimental effects on health (Kumaran and Karunakaran 2007; Alp and Aksu 2010). There are many plants that can be a good source of natural antioxidants. One of the effective natural antioxidant sources is known as *Urtica dioica* L. (Aksu 2003; Aksu and Kaya 2004; Gulcin *et al.* 2004; Exarchou *et al.* 2006; Karabacak and Bozkurt 2008; Modarresi-Chahardehi *et al.* 2012; Oz 2014; Ramtin *et al.* 2014). *U. dioica* L. contains sort of vitamins, minerals, different compounds such as carotenoids, flavonoids and alkaloids, and essential amino acids, and it also possesses antioxidant and antimicrobial activity (Aksu and Kaya 2004; Gulcin *et al.* 2004; Alp and Aksu 2010; Abdeltawab *et al.* 2012; Bisht *et al.* 2012; Aksoy Korpe *et al.* 2013; Orcic *et al.* 2014).

Plant extracts of *U. dioica* L. are being studied to prolong the shelf life of fermented meats (Aksu 2003; Aksu and Kaya 2004; Karabacak and Bozkurt 2008), beef meatballs (Oz 2014) and ground beef meats (Alp and Aksu 2010). However, no research has been carried out on the effect of

lyophilized *U. dioica* L. water extract (LUDWE) addition on the quality characteristics and shelf life of vacuum-packaged beef steaks during storage. Beef steaks are perishable fresh meat products because of their suitable nutritive properties, pH, a_w values and microorganisms on the surface of the sliced meat. Therefore, quality properties such as color, microbial counts and lipid oxidation of beef steaks can be changed during storage. It is possible to extend the shelf life of beef steaks by adding natural extracts including antioxidant and antimicrobial agents and by vacuum packaging. The present study was designed to examine the effects of 150, 300 and 500 ppm LUDWE during storage time (0, 3, 6, 9, 12, 15, 18 and 21 days) on the psychrotrophic, lactic acid, *Pseudomonas*, and Enterobacteriaceae bacteria counts, pH, thiobarbituric acid reactive substances (TBARS) and color values of vacuum-packaged beef steaks at 2 ± 0.5C.

MATERIALS AND METHODS

Materials

Beef *longissimus thoracis et lumborum* (LTL) muscles (Kauffman *et al*. 1990) were obtained from a local abattoir (EBK Slaughterhouse, Erzurum, Turkey) inspected by local authorities. *U. dioica* L. plants were collected from Rizekent, a village at the Erzurum province in Turkey. Packaging materials were taken from a manufacturing company (Cryovac BB3050 BAG, Sealed Air GmbH, D-22844, Norderstedt, Germany). PA/PE (Polyamide/Polyethylene, 15 × 25 cm, O_2 permeability 40 cm^3/m^2/day·atm·23C; CO_2 permeability 1,454 cm^3/m^2/day·atm·23C; N_2 permeability 24 cm^3/m^2/day·atm·23C; and water vapor permeability <3g/m^2/day·atm·23C) was used as packaging material.

Preparation of the LUDWE

Dried *U. dioica* L. leaves (30–35C, <2% moisture) were ground and sifted through a 14-in. sieve. About 20 g of this material was added to 400 mL of boiling water and mixed by a magnetic stirrer for 15 min, and filtered with Whatman No. 1 (GE Healthcare UK Limited, Amersham Place Little Chalfont, Buckinghamshire, HP7 9NA, UK). Then, it was frozen at −38C and lyophilized at −50C (Aksu 2003; Alp and Aksu 2010; Aksu and Ozer 2013).

Preparation of the Beef Steaks and Addition of the LUDWE

Two beef carcasses were used in this trial. One of the beef carcasses was used for replication 1, and other one was used for replication 2. After 24-h postmortem, LTL muscle was removed from both sides of two beef carcasses under hygienic conditions and they were kept at 4C until used. Then, muscles were sliced at 1 in. thickness (2.54 cm) and 64 beef steaks were obtained from one carcass. A total of 128 steak samples (64 steaks for replication 1, 64 steaks for replication 2) were used. Steaks obtained from one of the carcasses were randomly divided into four treatment groups and 18 steaks were used for each experiment of each replication (36 steak samples [18 for replication 1 + 18 for replication 2] vacuum packaged for each treatment group: control = 36 steaks, 150 ppm LUDWE = 36 steaks, 300 ppm LUDWE = 36 steaks, 450 ppm LUDWE = 36 steaks; total steak weight: 28 kg; for each treatment group: 7 kg). The first group of beef steaks was put into the packaging materials and they were immediately vacuum packed. The second group of beef steaks was prepared by adding 150 ppm LUDWE and packaged in vacuum. The third and fourth groups of beef steaks were prepared by 300 and 450 ppm LUDWE and then were vacuum packaged with vacuum packaging units (MULTIVAC Packaging Unit 300/16 Sepp. Haggenmuller D 87787, Wolfertschwenden, Germany).

Storage and Analysis

Vacuum-packaged beef steaks were stored at 2 ± 0.5C for 21 days. Color (L^*, a^* and b^*), TBARS values, psychrotrophic, lactic acid, *Pseudomonas* and Enterobacteriaceae bacterial counts were determined on the 0, 3rd, 6th, 9th, 12th, 15th, 18th and 21st days of the storage time, while the pH values were done on the 0, 3rd, 9th, 15th and 21st days. For each storage time, two vacuum-packaged steaks from each treatment group of each replication were analyzed.

Color Measurements

Color values were determined using a Minolta Colorimeter CR-400 (Illuminant D65, Ø11mm, Minolta Co, Osaka, Japan). The colorimeter was calibrated using a white standard plate prior to each use for values recorded on beef steaks. The color of the beef steaks was measured at four different locations across the surface, and the average for each beef steak was calculated.

Determination of TBARS Values

For TBARS analysis, 1 g of meat was taken from each beef steak and 6 mL of trichloroacetic acid (TCA) solution (7.5% TCA with 0.1% propyl gallate and 0.1% ethylenediaminetetraacetic acid) was added. It was homogenized with Ultra-turrax (Nach Prof. P. Willem, Janke and Kunkel KG, IKA 25 Werk, Staufen im Breisgau, Germany) for 15–30 s, and then the mixture was filtered using Whatman 1. One milliliter of 0.02 M thiobarbituric acid solution was added on 1 mL filtrate. The mixture was kept in a boiling water bath for 40 min and then it was cooled

(20C) and centrifuged at 2,000 × g for 5 min. Absorbance of each supernatant was measured at 532 nm (UV 160, Shimadzu, Kyoto, Japan). TBARS values were calculated as μmol malonaldehyde/kg of beef steaks (Tarladgis *et al.* 1962; Lemon 1975; Aksu 2009).

Determination of the pH Values

For pH value, 10 g of beef steak samples was homogenized with 100 mL of distilled water (IKA 25 Werk, Nach Prof. P. Willem, Janke and Kunkel KG), and then this homogenate was used for pH analysis. pH values were measured by a pH meter (Thermo Scientific, pH Benchtop, Orion 3 Star).

Microbiological Analysis

A sample of 25 g of beef steak was placed aseptically in a stomacher bag, 225 mL of physiological solution containing 0.85% NaCl was added, and then it was homogenized in a stomacher bag for 1 min. Serial dilutions were prepared and surface plate method was used for enumeration. Psychrotrophic bacteria were enumerated on plate count agar (Oxoid CM 0325, Hampshire, England) at 10C for 7 days. For *Pseudomonas* bacteria, CFC (cetrimide–fucidin–cephaloridine) selective agar supplement (Oxoid SR 0103), supplemented CFC agar (*Pseudomonas* agar base, Oxoid CM 0559, Hampshire, England) was used, and plates were incubated aerobically at 25C for 48 h. Enterobacteriaceae and lactic acid bacteria were incubated anaerobically on violet red bile dextrose agar (Oxoid CM 0485, Hampshire, England) and De Man Rogosa Sharpe agar (Oxoid CM 0361, Hampshire, England) at 30C for 2–3 days, respectively. Bacterial counts were expressed as colony-forming units per gram of beef steaks sample (cfu/g).

Statistical Analysis

The results were analyzed using the general linear model procedure of SPSS (1996) 12.0 for windows for a completely randomized block design, using two replicates. Differences among means were made using Duncan's multiple range test at a significance level of $P < 0.05$. The model included treatment (Control, LUDWE-150, LUDWE-300 and LUDWE-450) and storage time (0, 3, 6, 9, 12, 15, 18 and 21 days) as main effects and all their interactions. Means were separated using Duncan's multiple range test, and the results were shown as mean values ± standard error in the tables.

RESULTS AND DISCUSSION

The results of pH, TBARS, L^*, a^* and b^* in control and beef steaks with LUDWE are shown in Table 1. The level of LUDWE had a statistically significant effect ($P < 0.01$) on TBARS values of beef steak; the TBARS values of beef steaks with LUDWE were lower than the control samples ($P < 0.05$). As expected, storage time affected ($P < 0.01$) TBARS values (Table 1). The highest values were found at

TABLE 1. TBARS, pH, L^*, A^* AND B^* VALUES OF BEEF STEAKS STORED AT 2.0 ± 0.5C FOR 21 DAYS

	TBARS†	pH	L^* value	a^* value	b^* value
Treatment (T)					
Control	11.24 ± 0.40^{a}	5.63 ± 0.007^{a}	41.09 ± 0.42^{a}	17.05 ± 0.28^{a}	5.50 ± 0.23^{c}
150 ppm LUDWE	9.35 ± 0.18^{b}	5.62 ± 0.007^{b}	40.68 ± 0.41^{ab}	16.90 ± 0.24^{ab}	6.12 ± 0.22^{b}
300 ppm LUDWE	9.06 ± 0.15^{b}	5.62 ± 0.004^{b}	40.00 ± 0.39^{bc}	16.37 ± 0.26^{b}	6.51 ± 0.18^{b}
450 ppm LUDWE	8.49 ± 0.19^{b}	5.61 ± 0.04^{b}	39.45 ± 0.30^{c}	16.33 ± 0.23^{b}	7.51 ± 0.22^{a}
SEM	0.175	0.05	0.361	0.206	0.193
P	**	**	**	*	**
Storage time (day) (ST)					
0	8.10 ± 0.23^{c}	5.60 ± 0.003^{c}	38.67 ± 0.35^{c}	14.52 ± 0.21^{c}	5.32 ± 0.32^{c}
3	8.25 ± 0.25^{c}	5.61 ± 0.005^{bc}	39.05 ± 0.74^{bc}	15.65 ± 0.38^{b}	5.82 ± 0.24^{bc}
6	9.54 ± 0.31^{b}	–	40.22 ± 0.64^{ab}	15.79 ± 0.27^{b}	5.92 ± 0.30^{bc}
9	9.59 ± 0.34^{b}	5.61 ± 0.006^{bc}	40.50 ± 0.41^{ab}	16.82 ± 0.32^{a}	6.41 ± 0.36^{ab}
12	9.59 ± 0.28^{b}	–	40.70 ± 0.34^{a}	17.69 ± 0.32^{a}	6.74 ± 0.23^{a}
15	10.22 ± 0.32^{b}	5.62 ± 0.005^{b}	40.80 ± 0.44^{a}	17.57 ± 0.29^{a}	7.06 ± 0.31^{a}
18	10.01 ± 0.49^{b}	–	40.80 ± 0.50^{a}	17.61 ± 0.53^{a}	7.11 ± 0.36^{a}
21	11.00 ± 0.69^{a}	5.64 ± 0.008^{a}	41.69 ± 0.62^{a}	17.65 ± 0.27^{a}	6.87 ± 0.34^{a}
SEM	0.247	0.01	0.510	0.292	0.273
P	**	**	**	**	**
Interactions					
T × ST	**	NS	NS	**	NS

$^{a-c}$Means in the same column and the same section with the same letters are not significantly different at *$P < 0.05$.* $P < 0.05$; ** $P < 0.01$.† μmol malonaldehyde/kg.±, standard error; LUDWE, lyophilized *Urtica dioica* L. water extract; NS, nonsignificant; SEM, standard error of means; TBARS, thiobarbituric acid reactive substances.

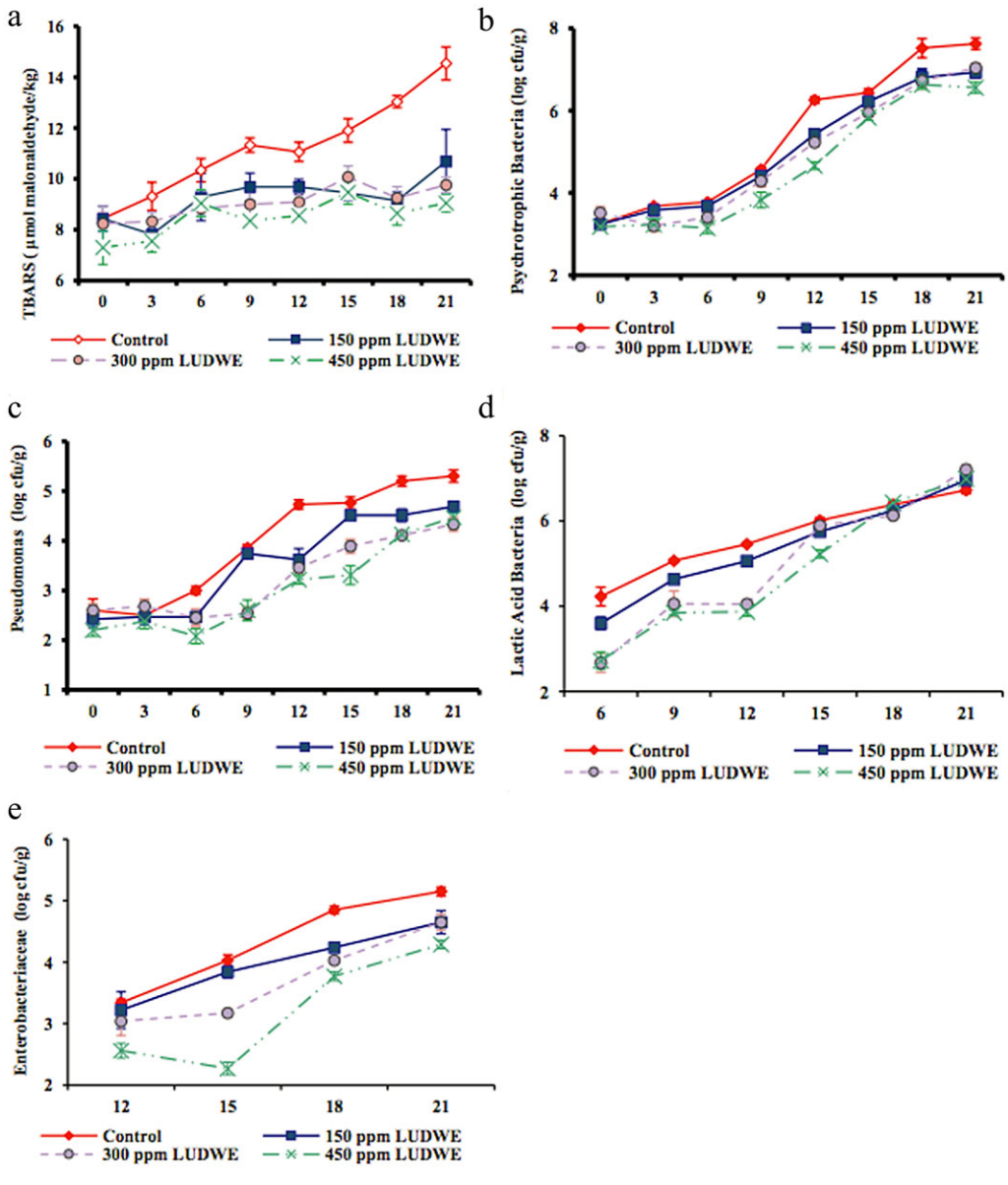

FIG. 1. CHANGES DURING STORAGE IN BEEF STEAK WITH LYOPHILIZED *URTICA DIOICA* L. WATER EXTRACT (LUDWE)
(a) Thiobarbituric acid reactive substances (TBARS) values. (b) Psychrotrophic bacteria counts. (c) *Pseudomonas* counts. (d) Lactic acid bacteria counts. (e) Enterobacteriaceae counts.

the end of storage time ($P < 0.05$). Treatment × storage time interaction also had a significant effect ($P < 0.01$) on TBARS values (Fig. 1a). As seen in Fig. 1a, the highest TBARS values were found in the control beef steaks, while the sample with 450 ppm LUDWE showed the lowest values during storage. Some researchers also reported that dried ground leaves of *U. dioica* L. and LUDWE had an antioxidant effect on the fresh meat and meat products. Alp and Aksu (2010) studied the antioxidant effects of LUDWE in the ground beef meats stored at 2 ± 0.5C for 14 days and found that TBARS values of ground beef decreased depending on the level of LUDWE. Similarly, other researchers also determined that TBARS values of the meatballs with LUDWE (Oz 2014) and of the fermented sausages with *U. dioica* L. (Karabacak and Bozkurt 2008) were lower than the samples without *U. dioica* L. Antioxidant properties of the *U. dioica* L. plant can be explained by the antioxidant activity and phenolic contents of the plant or plant extracts because inhibition of the lipid peroxidation is mainly related to phenolic compounds such as phenolic acid and flavonoids in the extract

of plants. Orcic *et al.* (2014) found that *U. dioica* L. extract is a rich source of phenolics such as 5-*O*-caffeoylquinic acid, rutin and isoquercitrin compounds with confirmed antioxidant. *U. dioica* L. extracts are also rich sources of flavonoid, alkaloid, phenol, saponin and tannin (Abdeltawab *et al.* 2012), carotenoids (Kudritsata *et al.* 1986; Guil-Guerreroa *et al.* 2003; Upton 2013) and chlorophylls (Hojnik *et al.* 2007). On the other hand, high oxygen concentrations in the package bring about lipid oxidation. With the elimination of oxygen, lipid oxidation is significantly reduced, thereby extending the shelf life of fresh meat and meat products. Vacuum packaging is the most common method of reducing the oxygen in the package. Gokoglu *et al.* (2011) reported that TBA values of vacuum-packaged beef steaks stored at 2.0 ± 0.5C increased from 0.22 ± 0.44 to 0.65 ± 0.01 mg malonaldehyde/kg during 0 and 14 days of storage. Similar TBARS values in vacuum-packaged beef steaks were also indicated by several researchers (Vitale *et al.* 2014; Bagdatli and Kayaardi 2015).

In the present study, the usage of lyophilized *U. dioica* L. water extract in beef steaks significantly affected the pH values ($P < 0.01$), and pH values were significantly lower in the 150, 300 and 450 ppm LUDWE groups than in the control group ($P < 0.05$). The highest values were found in the control group as average (5.63 ± 0.03; Table 1). Control pH values tended to increase to an average value of 5.61 during the first 3 days and the last 3 days of storage. At the end of storage (21 days), the average pH value (5.67) of the control beef steak was higher ($P < 0.05$) than the average of 5.62, 5.64 and 5.63 observed for beef steaks with 150, 300 and 450 ppm LUDWE, respectively. However, Aksu (2003) and Kaban *et al.* (2007, 2008) determined that pH values increased in dry fermented sausage with different levels (1–5%) of dried ground *U. dioica* L. leaves. On the contrary, in the present study, the pH values of beef steak samples with LUDWE were lower than the control beef steak samples. This can be explained as follows: (1) the 150–450 ppm LUDWE levels are very low to increase the pH value and (2) the antimicrobial effects of LUDWE on Enterobacteriaceae and *Pseudomonas* counts were showed during storage (Fig. 1c and e). Enterobacteriaceae and *Pseudomonas* bacteria have proteolytic activity; thus, high counts of these bacteria increase the pH values of fresh meats. On the other hand, pH values of vacuum-packaged, stored beef steaks increased during storage (Keun-Taik and Chan-Suk 2001; Katikou *et al.* 2005; Jose and Gómez 2012; Bagdatli and Kayaardi 2015).

The L^* and a^* values were negatively related to the treatment, while the b^* values were positively related to the treatment (Table 1). With increasing LUDWE in beef steaks, the L^* and a^* values decreased, but the b^* values increased ($P < 0.01$). The highest L^* and a^* values were determined in the control beef steaks ($P < 0.05$) while the b^* values were determined in beef steaks with 450 ppm LUDWE (Table 1). In the present study, the L^* and a^* values decreased because of LUDWE. Lyophilized *U. dioica* L. water extract is green; thus, the L^* and a^* values can decrease with LUDWE. A good natural green pigment source of *U. dioica* L. extracts was reported by Hojnik *et al.* (2007). Alp and Aksu (2010) also reported that the b^* value increased in the ground beef samples with LUDWE. In addition, storage time was significantly affected by the L^*, a^* and b^* values. During the first 9 days, the L^*, a^* and b^* values tented to increase, and then they did not statistically change (Table 1). Similar changes for color values during storage were also determined by Vitale *et al.* (2014) for beef meats, by Aviles *et al.* (2014) and Liu *et al.* (2003) for beef steaks, by Alp and Aksu (2010) and Aksu and Alp (2012) for ground beef.

Average psychrotrophic counts of control and other beef steaks with 150, 300 and 450 ppm LUDWE determined during storage time are presented in Table 2. The usage of LUDWE in beef steaks significantly affected the psychrotrophic counts ($P < 0.01$), and the values were lower in the 150, 300 and 450 ppm LUDWE groups than in the control group ($P < 0.05$). Furthermore, there were significant ($P < 0.01$) differences in psychrotrophic counts among beef steaks with LUDWE, and the lowest values were observed in beef steaks with 450 ppm LUDWE with a mean value of 4.67 ± 1.47 cfu/g. Treatment × storage time interactions also had a significant effect ($P < 0.01$) on the psychrotrophic counts, and the highest count occurred in the control beef steaks during storage time (Fig. 1b). However, there was further difference in the psychrotrophic counts after 9 days of storage. The lowest psychrotrophic counts were determined in the beef steaks with 450 ppm LUDWE during storage. During the 21st day of storage, the average psychrotrophic counts of the control beef steak (7.63 log cfu/g) were higher ($P < 0.05$) than the average of 6.93, 7.04 and 6.56 log cfu/g observed for beef steaks with 150, 300 and 450 ppm LUDWE as shown in Fig. 1b. Alp and Aksu (2010) determined that inhibition psychrotrophic bacteria in modified atmosphere-packaged ground beef stored at 2 ± 0.5C for 14 days were achieved using a 500 ppm LUDWE. Moreover, the authors reported that increasing the storage time and storage temperature progressively increased the psychrotrophic counts of different fresh meat and meat products (Capita *et al.* 2006; Rodas-González *et al.* 2011; Degirmencioglu *et al.* 2012; Jose and Gómez 2012). Therefore, inhibition of the psychrotrophic bacteria for meats during storage is very important to prevent spoilage. In the present study, determined results showed that LUDWE could be used as a good natural antimicrobial agent for vacuum-packaged beef steaks.

There was a significant ($P < 0.01$) change in *Pseudomonas* count in beef steaks with LUDWE and control. LUDWE

TABLE 2. PSYCHROTROPHIC, *PSEUDOMONAS*, LACTIC ACID BACTERIA AND ENTEROBACTERIACEAE COUNTS OF BEEF STEAKS STORED AT 2.0 ± 0.5C FOR 21 DAYS

	Psychrotrophic (log cfu/g)	*Pseudomonas* (log cfu/g)	Lactic acid bacteria (log cfu/g)	Enterobacteriaceae (log cfu/g)
Treatment (T)				
Control	5.26 ± 0.27[a]	3.99 ± 0.20[a]	5.66 ± 0.18[a]	4.34 ± 0.19[a]
150 ppm LUDWE	5.04 ± 0.25[b]	3.55 ± 0.16[b]	5.37 ± 0.23[b]	3.99 ± 0.16[b]
300 ppm LUDWE	4.92 ± 0.30[c]	3.25 ± 0.14[c]	5.00 ± 0.33[c]	3.72 ± 0.18[c]
450 ppm LUDWE	4.67 ± 0.26[d]	3.05 ± 0.15[d]	4.85 ± 0.32[d]	3.22 ± 0.22[d]
SEM	0.28	0.04	0.04	0.044
P	**	**	**	**
Storage time (day) (ST)				
0	3.30 ± 0.05[g]	2.45 ± 0.09[f]	<2.0	<2.0
3	3.44 ± 0.07[f]	2.50 ± 0.08[f]	<2.0	<2.0
6	3.51 ± 0.08[f]	2.50 ± 0.10[f]	3.30 ± 0.19[f]	<2.0
9	4.27 ± 0.08[e]	3.18 ± 0.17[e]	4.40 ± 0.14[e]	<2.0
12	5.39 ± 0.15[d]	3.76 ± 0.16[d]	4.61 ± 0.18[d]	3.04 ± 0.12[d]
15	6.11 ± 0.07[c]	4.12 ± 0.15[c]	5.74 ± 0.09[c]	3.33 ± 0.18[c]
18	6.79 ± 0.09[b]	4.49 ± 0.12[b]	6.29 ± 0.04[b]	4.22 ± 0.11[b]
21	6.95 ± 0.06[a]	4.70 ± 0.11[a]	6.97 ± 0.05[a]	4.69 ± 0.10[a]
SEM	0.04	0.056	0.049	0.044
P	**	**	**	**
Interactions				
T x ST	**	**	**	**

[a–g]Means in the same column and the same section with the same letters are not significantly different at * $P < 0.05$.** $P < 0.01$.±, standard error; LUDWE, lyophilized *Urtica dioica* L. water extract; SEM, standard error of means.

caused a significant ($P < 0.01$) decrease in *Pseudomonas* count of beef steaks, and the lowest values were determined in beef steaks with 450 ppm LUDWE as 3.05 ± 0.87 log cfu/g (Table 2). However, storage time caused a significant ($P < 0.01$) increase in *Pseudomonas* count, and the highest values were at the end of storage, as 4.70 ± 0.42 log cfu/g. *Pseudomonas* counts were lower ($P < 0.05$) in beef steaks with LUDWE samples than those of the control samples during storage (Fig. 1c). There was a significant difference among beef steaks with 150, 300 and 450 ppm LUDWE in *Pseudomonas* counts ($P < 0.05$), and the lowest counts during storage occurred in the beef steaks with 450 ppm LUDWE (Fig. 1c). *Pseudomonas* cause spoilage in the meat stored at low temperature. In the present study, it was observed that *Pseudomonas* in vacuum-packaged beef steaks can be inhibited by LUDWE. Gulcin *et al.* (2004), Alp and Aksu (2010), Modarresi-Chahardehi *et al.* (2012) and Ramtin *et al.* (2014) reported that the growth of *Pseudomonas* was prevented by *U. dioica* L. In contrast, Majd *et al.* (2003) determined that the inhibition effect on the *Pseudomonas* growth of *U. dioica* L. water extract was lower than that of *U. dioica* L. ethanol extract.

As shown in Table 2, LUDWE ($P < 0.01$) and storage time ($P < 0.01$) had a significant effect on the lactic acid bacteria counts. The lowest lactic acid bacteria counts were found in beef steaks with 450 ppm LUDWE ($P < 0.05$) as 4.85 ± 1.55 log cfu/g. Up to 6 days of storage, the average lactic acid bacteria count was <2.0 log cfu/g, and then this count tented to increase (Fig. 1d). During the 6th, 9th, 12th and 15th days of storage, lactic acid bacteria counts of control beef steaks were higher ($P < 0.05$) than the beef steaks with 150, 300 and 450 ppm LUDWE, but there was no significant difference during the 18th–21st days for control and with LUDWE beef steak samples (Fig. 1d). Aksu and Kaya (2004) reported similar results in their study related to fermented meat, that the lactic acid bacteria count was lower in the groups with *U. dioica* L. than that of the control group. However, Gokoglu *et al.* (2011) observed that lactic acid bacteria count in vacuum-packaged beef steaks during storage at 4C for 14 days increased from 3.53 ± 0.04 to 8.10 ± 0.03 log cfu/g.

Enterobacteriaceae counts were significantly different for treatment ($P < 0.01$) and storage time ($P < 0.01$) (Table 2). In the present study, the lowest Enterobacteriaceae counts were in the 450 ppm LUDWE added beef steak samples, and antimicrobial effect on Enterobacteriaceae count of LUDWE was dependent on the level of addition (Table 2). Enterobacteriaceae counts in both control and the 150, 300 and 450 ppm LUDWE beef steak samples were below the detection level (<2.0 log cfu/g) until the 12th day of storage. The values for the 12th, 15th, 18th and 21st days were significantly different as shown in Fig. 1e. Between 12 and 21 days of storage, the highest counts were found in the beef steaks without LUDWE, while the lowest counts were

determined in the 450 ppm LUDWE added beef steaks (Fig. 1e). Aksu and Kaya (2004) determined that dried ground *U. dioica* L. leaves had an antimicrobial effect on the Enterobacteriaceae counts of fermented meats. Similarly, Alp and Aksu (2010) found that Enterobacteriaceae counts of ground beef decreased depending on the level of LUDWE.

CONCLUSION

This work showed that LUDWE could be used as a good natural antioxidant and antimicrobial agent in fresh meat products such as beef steaks. It was observed that psychrotrophic bacteria, lactic acid bacteria, *Pseudomonas* and Enterobacteriaceae could be inhibited by LUDWE at a maximum of 450 ppm level. Especially, with the increasing levels of LUDWE in beef steaks, the counts of spoilage microorganism decreased. Spoilage of fresh meats occurs when total aerobic bacteria counts reach 10^7 cfu/g. Thus, spoilage of the vacuum-packaged beef steaks with LUDWE was delayed for 3 days (for 150 and 300 ppm) and >3 days (for 450 ppm) according to the determined psychrotrophic bacteria counts in samples.

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