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ORIGINAL ARTICLE

Effects of lyophilized black carrot (*Daucus carota* L.) water extract on the shelf life, physico-chemical and microbiological quality of high-oxygen modified atmosphere packaged (HiOx-MAP) ground beef

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Abstract In the present study, firstly, various properties of lyophilized water extracts (LBCWE) produced from fresh black carrot were determined. LBCWE was observed to be a rich source of monomeric anthocyanins (1188.40 ± 17.38 mg C3G/100 g; n = 4) and phenolics (2733.83 ± 17.78 mg GAE/100 g, n = 4). Secondly, ground beef containing LBCWE (Control, 100, 200 and 300 ppm) and packaged in HiOx-MAP (80% O_2 + 20% CO_2) was evaluated in terms of lipid oxidation, metmyoglobin (MetMb), color, pH and microbial counts during storage at 2.0 ± 0.5 °C for 12 days. By increasing level of LBCWE, the pH, lipid oxidation, MetMb and microbial counts were decreased ($P < 0.01$). The LBCWE significantly affected the color and microbial count parameters ($P < 0.01$). The highest redness and lowest microbial growth during storage was in the 300 ppm LBCWE group ($P < 0.05$). On the 12th day of storage, *Pseudomonas* and *Enterobactericeae* decreased 1.24 log and 1.46 log units in this group according to control. The shelf life of ground beef can be extended by 3 days with MAP + 300 ppm extract application.

Keywords Ground beef · Black carrot extract · HiOx-MAP · Metmyoglobin · Lipid oxidation · Microbial quality

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Introduction

Ground beef is among the foods with high risk group in terms of food safety, as it is an ideal substrate for the growth of various spoilage and pathogenic microorganisms. The shelf life of ground beef is short due to the rapid chemical, microbiological and physical reactions in terms of nutritional composition and structural properties. Therefore, colour, lipid and protein oxidation and microbial load are the most important quality criteria for ground meat. High oxygen permeable packaging, vacuum packaging, low or high O_2 content modified atmosphere (MAP) are used to protect these quality criteria (Zhou et al. 2010; Rogers et al. 2014; McMillin 2017). In MAP, depending on the composition of the atmosphere to be used, the shelf life is prolonged and the color stability increases. MAP with 70–80% oxygen is widely used and the oxygen required to maintain color stability is more than 55% (Lindahl 2011). Despite the advantages of MAP application with high oxygen levels such as providing bright cherry red color (oxymoglobin), excess oxygen accelerates lipid oxidation and eventually causes undesired dark red color formation and flavor disorders (Esmer et al. 2011; McMillin 2017; Bonny et al. 2017). The use of synthetic colorants and antioxidants has decreased due to their negative effects on human health and the use of plant extracts obtained from natural sources has become widespread in recent years (Zamuz et al. 2018; Aksu et al. 2020a, 2020b; Prommachart et al. 2020). Plant extracts rich in anthocyanins and phenolic substances are recognized as the best natural ingredients that prevent oxidation in many foods and provide bright red color formation (Espin et al. 2000; Alp and Aksu 2010; Aksu et al. 2020c). Studies reveal that black carrot has a wide range of total anthocyanin and phenolic content and has high levels of antioxidant activity (Algarra

et al. 2014; Assous et al. 2014; Mizgier et al. 2016). Due to these properties, the extract of black carrot is a good colorant (Kammerer et al. 2004; Assous et al. 2014), antioxidant (Algarra et al. 2014; Mizgier et al. 2016) and has antimicrobial properties on some pathogens (Degirmenci et al. 2012).

Studies to determine the properties of black carrot lyophilized water extracts (LBCWE) are limited, and no research has been found on the effects of these extracts on ground beef quality in HiOx-MAP. The objectives of the present study were: (1) to determine of various properties of LBCWE, (2) to determine of the effects of LBCWE (0, 100, 200 and 300 ppm) on the physico-chemical and microbiological quality of HiOx-MAP (80% O_2 + 20% CO_2) ground beef during storage at 2 ± 0.5 °C for 12 days.

Material and methods

Material

The ground beef used in the research was obtained from the Erzurum Meat and Milk Board Institution and fresh black carrots were obtained from the markets in the Erzurum, Turkey.

Obtaining black carrot lyophilized water extracts (LBCWE)

The black carrots were cleaned with water and shredded in a kitchen blender after the wash water was filtered. Subsequently, 400 mL of hot water was added to 20 g of crushed carrots and homogenized with an Ultra-Turrax. The homogenate obtained was kept in a magnetic stirrer for 15 min and filtered through Whatman No: 1 filter paper. The filtrate was concentrated by rotary evaporator, then frozen at −38 °C and lyophilization took place at −50 °C. The extracts obtained were kept at refrigerator temperature until used (Alp and Aksu 2010).

Addition of extracts to ground beef, packaging and storage

Ground beef (total 10 kg for one repeat) was divided into four groups and then ground beef samples for each treatment group were divided into 250 g pieces each. First group ground beef (10 pieces of 250 g) were placed in packaging bags and packaged in a modified atmosphere (HiOx-MAP, 80% O_2 + 20% CO_2), and this group was used as a control group. The 100 ppm extract was added to the second group of ground beef (10 pieces of 250 g), and 200 ppm and 300 ppm extracts were added to the third (10 pieces of 250 g) and fourth group (10 pieces of 250 g) of ground beef. The prepared samples were then manually mixed and packaged in HiOx-MAP. For MAP process, a Multivac vacuum packing unit (Multivac 300/16 Sepp. Haggenmuller D 87,787 Wolgertschwenden, Germany) was used, and polyamide/polyethylene was used as packaging materials (PA/PE, 15 × 25 cm, O_2 permeability 40 cm^3/m^2/day.atm. 23 °C; CO_2 permeability 1.454 cm^3/m^2/day.atm.23 °C; N_2 permeability 24 cm^3/m^2/day.atm. 23 °C; and water vapor permeability < 3 g/m^{2-}/day.atm.23 °C). All of the ground beef samples were stored at 2 ± 0.5 °C for 12 days. The trial repeated twice and analyzes on each storage day were performed in triplicate.

Analysis

Extract analysis

The amount of total phenolic substance of LBCWE was determined according to the method given by Singleton et al. (1999), and results are given as mg gallic acid equivalents (GAE) /100 g. The metal chelation activity was analyzed according to the procedure given by Bursal and Gülçin (2011), and results were calculated as mg BHA/100 g, mg BHT/100 g and mg Tocopherol/100 g. DPPH* (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was performed by applying the method given by Blois (1958), and the results were calculated in mmol trolox equivalents (TE)/100 g and IC_{50} (μg). The total monomeric anthocyanin content of LBCWE was determined using the pH differential method given by Lee et al. (2005), and results were calculated as mg cyanidine 3-glucoside (C3G)/ 100 g.

Determination of pH value and titration acidity

The pH and titration acidity values of LBCWE were determined according to the method given in Cemeroğlu (2010). In measuring of the pH value of the ground beef, 10 g of the ground beef were weighed parallel to each other, after adding 100 mL of distilled water, the mixture was homogenized with an Ultra-Turrax for 1 min. Then, the pH values of the mixtures were determined by using a pH meter calibrated with pH 4.0 and 7.0 buffer solutions.

Determination of colour values

The L* (lightness), a* (redness), and b* (yellowness) color values of LBCWE and ground beef samples according to the Commission Internationale de L'Eclairage (CIE) were

measured using the Minolta (CR-400, Minolta Co, Osaka, Japan) colorimeter with an 11-mm diameter illumination area, D65 illuminator and 2° standard observer conditions. Nine repeated measurements were taken for each sample on each storage day. The device was calibrated with a standard calibration plate before color measurements.

Determination of thiobarbituric acid reactive substance (TBARS) value

The TBARS values of the ground beef were determined according to the method given by Lemon (1975), but the method was modified. According to method, 6 mL of TCA solution (7.5% TCA, 0.1% EDTA, 0.1% propyl gallate; 1 g propyl gallate dissolved in 3 ml ethanol) was added on each 1 g of ground beef samples. The mixture was homogenized with an Ultra-Turrax for 15–30 s, then filtered through filter paper (Whatman 1). The method was modified at this stage. Because, there were different pinkish/reddish hues caused by extracts added to ground beef. These pink tints in the filtrates increased the absorbance value of the samples taken from ground beef with extract. Contrary to the pinking that occurs in sample filtrates, in procedures of this analysis, the desired pinking to determine the oxidation level is only the color change that occurs with the application of temperature after the addition of TBA solution to the filtrate obtained from the samples. For this reason, the absorbance (A_1) of the pink/red colored filtrates obtained from ground beef samples with extract were measured before adding TBA solution. This absorbance value was subtracted from the total absorbance value (A_2) of the solution containing filtrate + TBA and malonaldehyde amount was calculated. For analysis, one mL of the obtained filtrate was taken into a tube and 1 mL of 0.02 M thiobarbituric acid (TBA) solution was added. These mixtures were kept in boiling water bath for 40 min, cooled under tap water for 5 min and then centrifuged at 2000 × g for 5 min. The all absorbance of the samples was measured against blank at 532 nm. The blank was prepared by mixing one mL of TCA extract and one mL of TBA solution, and the steps applied for the samples were performed in the same way. A_2-A_1 was used for each sample absorbance. TBARS value was calculated as mg malonaldehyde/kg tissue.

Determination of the ratio of metmyoglobin

Determination of the ratio of metmyoglobin of ground beef was analyzed according to the procedure given by Kannan et al. (2001). 25 mL of ice-cold phosphate buffer (pH 6.8, 40 mM) was added onto five grams of ground beef samples. The mixture homogenized using an Ultra-Turrax for 10 s at 13,500 rpm was centrifuged at 4 °C and the supernatant was filtered with Whatman No: 1. The metmyoglobin (MetMb) ratio was calculated as percentage (%) according to the formula illustrated below:

$$\text{MetMb}\ (\%) = [1.395 - (A_{572} - A_{700})/(A_{525} - A_{700})] \times 100$$

Where A_{700}, A_{572} and A_{525} were the absorbances of the filtrate at 700 nm, 575 nm and 525 nm, respectively.

Microbiological analysis

Plate Count Agar (PCA) was used for total aerobic mesophilic and psychotrophic bacteria count, and incubated aerobically for 48 h at 37 °C for total aerobic mesophilic bacteria and 7 days at 10 °C for the total psychrotrophic bacteria count. Mannitol Salt Phenol Red Agar was used for *Micrococcus*/*Staphylococcus* count, and plates were incubated aerobically for 48 h at 30 °C. The count was determined by considering the catalase (+) cocci. For determination of the lactic acid bacteria counts, the MRS (de Man Rogosa Sharpe) Agar was used and incubated for 48 h at 30 °C under anaerobic conditions (Anaerocult A, Merck). At the end of the incubation, catalase test was performed and the count of lactic acid bacteria was determined by considering the catalase (-) colonies. CFC Agar (*Pseudomonas* Agar Base-Oxoid CM 0559) medium prepared by adding CFC Selective Agar Supplement (Oxoid SR 0103) was used to determine the counts of *Pseudomonas*. Inoculated petri dishes were incubated at 25 °C for 48 h in aerobic conditions. At the end of incubation, oxidase test was applied to the colonies and the counts of *Pseudomonas* were determined by counting oxidase (+) colonies. For determination of the *Enterobacteriaceae* counts, VRBD Agar (Violet Red Bile Dextrose) was used and inoculated petri dishes were incubated at 30 °C for 48 h in anaerobic conditions (Anaerocult A, Merck). At the end of incubation, the counts of red colonies larger than 1 mm was counted and the *Enterobacteriaceae* count was determined.

Statistical analysis

The model included LBCWE levels (Control, 100, 200 and 300 ppm) and storage time (1st, 3rd, 6th, 9th and 12th days) as main effects and all their interactions. The research was carried out with two replications according to the completely random block design. Variance analysis was performed on the data by using SPSS 22.0 statistical package program, taking into account the factors and their interactions as fixed effects and replication as a random effect. The mean of the main variation sources and significant differences were compared with Duncan's Multiple

Range Test ($\alpha = 0.05$). The mean, standard deviation and standard error of the mean (SEM) were calculated for each variable and shown in tables.

Results and discussion

Results of lyophilized black carrot water extracts (LBCWE)

Total monomeric anthocyanin content of LBCWE determined as 1188.40 ± 17.38 mg C3G/100 g and was quite high than many research results (Montilla et al. 2011; Algarra et al. 2014). Anthocyanins are important compounds desired in extracts. Anthocyanins not only improve the color properties of the foods or food raw materials to which they are added, but also increase the oxidative stability of foods and are important components for human health (Espin et al. 2000; Assous et al. 2014). The high total phenolic substance (2733.83 ± 17.78 mg GAE/100 g), metal chelating and DPPH* radical scavenging activities of the extracts are parameters that show its high antioxidant properties. Total phenolic content of the different black carrot varieties was reported as 17.9–75.3 mg GAE/100 g fresh weight by Montilla et al. (2011). In the present research, the metal chelating activity values calculated as BHA, BHT and tocopherol were 80.39 ± 3.0 mg BHA/100 g, 151.85 ± 6.42 mg BHT/100 g and 26.80 ± 1.13 mg Tocopherol/100 g, respectively. DPPH* radical scavenging activity was 43.83 ± 0.32 mmol TE/100 g and 225.49 ± 2.22 IC_{50} (μg). These values are much higher in fresh tissue determined by Algarra et al. (2014). The mean L*, a* and b* values of extracts were measured as 18.27 ± 0.13, 2.78 ± 0.25 and −0.69 ± 0.03, respectively, and pH and titratable acidity values were determined as 5.89 ± 0.02 and 0.32 ± 0.03% citric acid. Although the anthocyanin content in the composition of the extracts is high, the low a* value that expresses redness is due to the high extract pH and the low acidity value.

Results of ground beef treated with extract and packaged in modified atmosphere

pH values

Extract levels, storage time, and extract levels x storage time interaction had a significant ($P < 0.01$) effect on pH values of ground beef (Table 1). Figure 1a shows the interaction of extract levels x storage time on pH values of ground beef. During storage, the pH values of the LBCWE-added groups were lower than those of the control samples without extract, this decrease was dependent on the increasing extract level. There was a significant difference ($P < 0.05$) between the treatment groups at all storage times except day 1, where there was no difference ($P > 0.05$) between the 200 ppm and 300 ppm LBCWE groups. The highest pH value was determined in control samples on day 12 of storage. The pH values of ground beef samples with extract reached the highest value on the 3rd day of storage and lower values were determined in the following days (Fig. 1a). The average pH values of 100, 200 and 300 ppm extract added ground beef samples decreased by 0.16, 0.27 and 0.33 units compared to the control (Table 1). The pH values of ground beef samples decreased with LBCWE application, this decline was depend on the extract level and was also observed during storage. Three factors are thought to be effective in this reduction. First, the acidity of the extract used was effective in the pH decline. Secondly, the effectiveness of CO_2 in the composition of the modified atmosphere increased depending on the level of the extract and therefore the pH of samples with extract decreased more than the control group. The third was to decrease the pH by providing more organic acid formation depending on the microbial growth in the samples with extract. However, higher pH values in the control samples, although they are kept under the same conditions (80% O_2 + 20% CO_2 and 2 ± 0.5 °C), results from the increase in the amount of substances such as ammonia. Because the oxidation of meat proteins increases with the growth of proteolytic bacteria and these substances are formed as end-products of oxidation. The fact that the pH values of the extract-added ground meat products were lower than the control during storage resulted from the antimicrobial effect of the extracts on these bacteria. Another group of bacteria that causes the pH to change when storing fresh meat at low temperatures is lactic acid bacteria. In this context, especially in the control group samples, the pH value decreased noticeably until the 9th day after the 3rd day of storage, due to the increase in the counts of lactic acid bacteria (Fig. 1a).

TBARS values

The presence of oxygen in the HiOx-MAP increases lipid oxidation in fresh meat and meat products and reduces product quality. It has been emphasized that by using antioxidants in HiOx-MAP, lipid oxidation can be prevented and shelf life of ground beef can be extended (Alp and Aksu 2010; Aksu and Alp 2012).

In this study, a significant extract levels × storage time interaction ($P < 0.01$) was observed for TBARS values of ground beef samples with or without LBCWE (Table 1; Fig. 1b). The lowest TBARS values were determined in all samples with extract until the 6th day of

Table 1 Mean values and Duncan Multiple Comparison Test results ($p < 0.05$) of pH, TBARS, MetMb and colour (L*, a* and b*) values of HiOx-MAP ground beef with different levels LBCWE during 12 days of storage at 2 ± 0.5 °C (n = 4)

	pH	TBARS (mg MDA/kg)	MetMb (%)	Colour Values		
				*L**	*a**	*b**
Extract (LBCWE) Levels (EL)						
Control	5.65 ± 0.10^{a}	2.83 ± 1.33^{a}	42.74 ± 17.00^{a}	49.07 ± 1.38^{a}	19.02 ± 3.85^{c}	13.36 ± 0.94^{a}
100 ppm	5.49 ± 0.05^{b}	2.80 ± 1.59^{a}	38.93 ± 15.24^{b}	48.75 ± 1.17^{ab}	19.74 ± 3.22^{b}	13.08 ± 1.03^{ab}
200 ppm	5.38 ± 0.07^{c}	2.76 ± 1.71^{a}	38.39 ± 15.46^{b}	48.48 ± 1.47^{ab}	20.17 ± 2.84^{ab}	13.02 ± 1.05^{ab}
300 ppm	5.32 ± 0.08^{d}	2.48 ± 1.44^{b}	36.67 ± 13.17^{c}	47.81 ± 1.57^{b}	20.58 ± 2.85^{a}	12.79 ± 1.08^{b}
SEM	0.006	0.025	0.566	0.323	0.248	0.161
P	**	**	**	NS	**	NS
Storage time (days) (ST)						
1	5.47 ± 0.11^{b}	0.39 ± 0.22^{e}	24.00 ± 2.95^{e}	49.89 ± 0.94^{a}	24.47 ± 1.15^{a}	14.57 ± 0.87^{a}
3	5.54 ± 0.11^{a}	1.72 ± 0.22^{d}	25.90 ± 3.30^{d}	48.36 ± 1.19^{b}	21.43 ± 0.99^{b}	13.25 ± 0.45^{b}
6	5.39 ± 0.12^{d}	3.21 ± 0.20^{c}	33.34 ± 4.71^{c}	48.40 ± 1.53^{b}	19.53 ± 0.58^{c}	12.55 ± 0.55^{bc}
9	5.43 ± 0.12^{c}	3.97 ± 0.32^{b}	54.68 ± 5.96^{b}	48.22 ± 1.08^{b}	18.21 ± 0.63^{d}	12.37 ± 0.33^{c}
12	5.47 ± 0.22^{b}	4.30 ± 0.21^{a}	58.01 ± 2.37^{a}	47.78 ± 1.62^{b}	15.76 ± 2.01^{e}	12.57 ± 0.80^{bc}
SEM	0.007	0.028	0.657	0.362	0.277	0.180
P	**	**	**	**	**	**
Interactions						
EL x ST	****	****	****	**NS**	**	NS

** $P < 0.01$; NS: Non-significant

a-e Means in the same column and in the same section having the same letters are not significantly different at $P > 0.05$

LBCWE: Lyophilized black carrot water extract

SEM: Standard Error of Means

storage compared to the control. However, on the 9th and 12th days of storage, only 300 ppm extract added ground beef had a lower TBARS value than control. It was determined that lipid oxidation of HiOx-MAP ground beef could be reduced/prevented by adding 300 ppm LBCWE (Table 1; Fig. 1b). The antioxidant capacities of black carrots were found to be high due to the high amount of anthocyanins and phenolic substances in both the results of the extract analysis used in this study and in many research results, but their usage levels are important (Stintzing et al. 2002; Kammerer et al. 2004; Algarra et al. 2014; Assous et al. 2014). Therefore, it was concluded that 300 ppm and higher amount of extract should be used to prevent lipid oxidation in HiOx-MAP. In a study conducted by Alp and Aksu (2010) determined that the 500 ppm *Urtica dioica* L. extract is suitable level for preventing lipid oxidation in ground beef. Zheng and Wang (2001) stated that phenolic compounds contained in natural antioxidants prevent oxidation by neutralizing free radicals by giving a hydrogen or electron to free radicals. Alp and Aksu (2010) and Aksu and Alp (2012) determined that high O_2 levels used for fresh meat packaged increased lipid oxidation during storage. Zamuz et al. (2018) reported that the TBARS value increased from 0.40 to 5.79 mg MDA/kg samples during storage (2 ± 1 °C, 18 days). Bonny et al. (2017) reported that TBARS values of beef meat increased by 6–8 mg MDA/kg under MAP (80% O_2 + 20% CO_2, 4 °C, 21 days).

Metmyoglobin values

The metmyoglobin (MetMb) formation percentage of ground beef was significantly ($P < 0.01$) affected by the LBCWE levels, storage time and extract levels x storage time interaction (Table 1). As seen in Fig. 1c, the percentage of MetMb increased in all groups during 12 days of storage, the increase in the control without LBCWE was greater. Generally, the highest and lowest MetMb% were determined in the control and 300 ppm LBCWE-added groups. No difference was observed between the treatment groups until day 6 of storage ($P > 0.05$), while the MetMb% values of ground beef samples containing extract were lower in the following days compared to the control group ($P < 0.05$). Considering consumer preferences, the 40% threshold reported by Greene et al. (1971) was exceeded on the 6th day in the control group, whereas

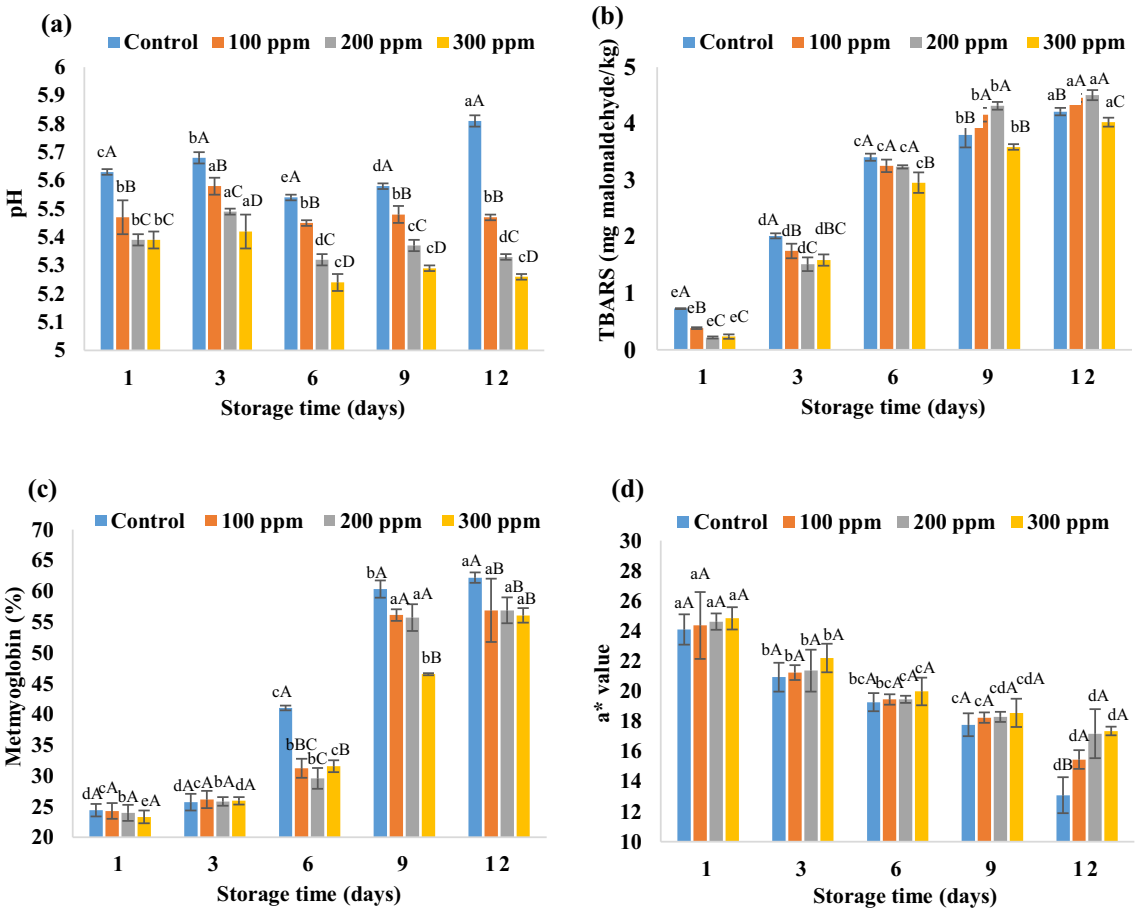

Fig. 1 The interaction of different levels of lyophilized black carrot water extract x storage time on **a** pH, **b** TBARS, **c** Metmyoglobin and **d** a* colour values (n = 4)

MetMb values were found above this limit value after 9 days in all LBCWE-treated groups (Fig. 1c). HiOx-MAP is a suitable method to provide the red color desired by the consumers, but long exposure of the meat to oxygen causes oxidation of oxymyoglobin (Oxy-Mb) and rapid formation of metmyoglobin resulting as discoloration (Rogers et al. 2014). Lipid peroxidation products and free radicals are related with discoloration caused by oxidation of Oxy-Mb to metmyoglobin (Liu et al. 2010). The presence of a thick Oxy-Mb layer that masks the underlying MetMb layer on the meat surface explains the prolonged color stability of the MAP compared to air. At least 55% oxygen content is needed to maintain the color stability and MAP with 70–80% oxygen is widely used (Lindahl 2011). Therefore, it is necessary to use antioxidants to prevent lipid and pigment oxidation in MAP meat. Many studies have shown that the formation of MetMb, which is highly correlated with lipid oxidation, can be reduced by the use of antioxidants (Liu et al. 2015; Zamuz et al. 2018). As can be seen from the current results, LBCWE delayed the formation of metmyoblobin compared to control ground beef with its rich content that contributes to metal chelating and antioxidant activities.

In present study, MetMb results of ground beef showed high correlation with TBARS ($r = 0.867$, $P < 0.01$) and redness value ($r = -0.845$, $P < 0.01$) (data not shown). In addition, a high relationship was observed with *Pseudomonas* ($r = 0.899$, $P < 0.01$) counts, which are known to support the formation of metmyoglobin by decreasing the oxygen partial pressure (Zhang et al. 2011). The removal of oxygen by internal factors to achieve low oxygen partial pressures takes place by the consumption of oxygen, which results in the oxidation of Oxy-Mb to the metmyoglobin (Mancini and Hunt 2005). The increase observed in *Pseudomonas* counts especially after the 6th day of storage explains the relationship with this situation (Tables 2, 4). Therefore, the current study showed that the addition of LBCWE to ground beef stored in HiOx-MAP contributes to preventing the formation of metmyoglobin by reducing *Pseudomonas* counts as well as delaying oxidation due to

Table 2 Mean values and Duncan Multiple Comparison Test results ($p < 0.05$) of microbial counts of HiOx-MAP ground beef with different levels LBCWE during 12 days of storage at 2 ± 0.5 °C (n = 4)

	Total aerobic mesophilic bacteria (log CFU/g)	Psychrotrophic bacteria (log CFU/g)	*Micrococcus/ Staphylococcus* (log CFU/g)	Lactic acid bacteria (log CFU/g)	*Pseudomonas* (log CFU/g)	*Enterobacteriacea* (log CFU/g)
Extract (LBCWE) Levels (EL)						
Control	5.53 ± 1.32^{a}	5.48 ± 1.03^{a}	3.85 ± 0.23^{a}	4.26 ± 1.03^{a}	4.93 ± 0.87^{a}	2.92 ± 0.47^{a}
100 ppm	5.13 ± 1.07^{b}	5.26 ± 1.03^{b}	3.69 ± 0.19^{b}	4.00 ± 1.00^{b}	4.69 ± 1.02^{b}	2.34 ± 0.34^{b}
200 ppm	4.92 ± 0.97^{c}	4.91 ± 0.85^{d}	3.66 ± 0.09^{b}	3.89 ± 1.00^{bc}	4.18 ± 0.83^{d}	2.32 ± 0.32^{b}
300 ppm	4.90 ± 0.86^{c}	5.06 ± 0.69^{c}	3.73 ± 0.15^{b}	3.84 ± 0.96^{c}	4.31 ± 0.49^{c}	2.19 ± 0.14^{c}
SEM	0.055	0.041	0.032	0.049	0.041	0.036
P	**	**	**	**	**	**
Storage Time (days) (ST)						
1	3.90 ± 0.30^{e}	4.13 ± 0.21^{e}	3.71 ± 0.17^{b}	2.83 ± 0.14^{e}	3.69 ± 0.20^{e}	2.19 ± 0.15^{d}
3	4.36 ± 0.29^{d}	4.60 ± 0.19^{d}	3.71 ± 0.08^{b}	3.29 ± 0.19^{d}	3.86 ± 0.24^{d}	2.31 ± 0.43^{c}
6	4.99 ± 0.57^{c}	4.99 ± 0.59^{c}	3.64 ± 0.12^{b}	3.83 ± 0.42^{c}	4.35 ± 0.63^{c}	2.33 ± 0.32^{c}
9	5.67 ± 0.43^{b}	5.73 ± 0.52^{b}	3.75 ± 0.11^{b}	4.54 ± 0.31^{b}	5.10 ± 0.43^{b}	2.46 ± 0.22^{b}
12	6.60 ± 0.52^{a}	6.44 ± 0.28^{a}	3.86 ± 0.30^{a}	5.49 ± 0.28^{a}	5.64 ± 0.54^{a}	2.92 ± 0.55^{a}
SEM	0.061	0.046	0.035	0.055	0.046	0.040
P	**	**	**	**	**	**
Interactions						
EL x ST	**	**	**	**	**	**

* $P < 0.05$; ** $P < 0.01$

a-e Means in the same column and in the same section having the same letters are not significantly different at $P > 0.05$

LBCWE: Lyophilized black carrot water extract

SEM: Standard Error of Means

its free radical scavenging and metal chelating activities. Yang et al. (2020) defined *Pseudomonas* as the most important bacteria that causes meat discoloration under HiOx-MAP conditions. Researchers also reported that *Pseudomonas* gradually prevailed over other bacteria from day 10 during storage of beef steak at 2 °C for 20 days, and promotes the discoloration of meat by increasing the oxygen consumption rate. Kiyimba et al. (2019) reported that high oxygen (80% O_2) conditions increase the Oxy-Mb oxidation caused by HNE (4-hydroxyl-2-nonenal), a lipid oxidation product that can increase Oxy-Mb oxidation, compared to atmospheric partial pressure (20% O_2) condition.

Colour values

Storage time had a significant effect ($P < 0.01$) on the L* and b* color values of ground beef, while the effects of LBCWE levels and the two-way interaction of these parameters were insignificant ($P > 0.05$). On the other hand, the a* value expressing redness was significantly ($P < 0.01$) affected by extract levels, storage time and extract levels x storage time interaction (Table 1). L* values of ground beef samples generally decreased during the storage period, while the highest decrease compared to other treatments was observed in 300 ppm LBCWE-added group. Until day 12, there was no significant difference ($P > 0.05$) between the b* values of the treatment groups, but the lowest b* value at the end of storage was detected in ground beef with 300 ppm extract ($P < 0.05$). The L*and b* values generally decreased during 12 days of storage (Table 3).

In Fig. 1d, the interaction of extract level x storage time ($P < 0.01$) on redness is presented. The addition of LBCWE increased a* values of ground beef samples stored in HiOx-MAP conditions compared to the control, and this increase was depend on the extract level (Table 1, Fig. 1d). The high oxygen content used in HiOx-MAP and the color substances such as anthocyanins in the composition of the extract used are two effective factors in increasing the color. Additionally, pH is thought to be effective in increasing color in ground beef samples with extract. Because, the effectiveness of anthocyanins increases at low pH levels and accordingly the red color density increases. The average pH values of the ground beef samples with extract were lower than the control group samples

Table 3 Colour (L*, a* and b*) values of HiOx-MAP ground beef with different levels LBCWE during 12 days of storage at 2 ± 0.5 °C (n = 4)

	Extract Levels	Storage time (days)					SEM
		1	3	6	9	12	
*L**	Control	50.10 ± 1.29^{aA}	48.62 ± 0.76^{aA}	49.02 ± 1.54^{aA}	48.43 ± 1.79^{aA}	49.20 ± 1.74^{aA}	0.723
	100 ppm	49.58 ± 1.22^{aA}	48.13 ± 2.01^{aA}	49.18 ± 0.25^{aA}	48.41 ± 0.24^{aA}	48.48 ± 1.32^{aAB}	
	200 ppm	49.89 ± 1.05^{aA}	48.36 ± 1.01^{abA}	48.81 ± 1.90^{abAB}	48.30 ± 0.93^{abA}	47.04 ± 1.45^{bAB}	
	300 ppm	49.98 ± 0.61^{aA}	48.34 ± 1.42^{abA}	46.58 ± 0.25^{bcB}	47.74 ± 1.34^{bcA}	46.40 ± 0.45^{cB}	
*a**	Control	24.09 ± 1.01^{aA}	20.92 ± 0.95^{bA}	19.26 ± 0.61^{bcA}	17.76 ± 0.76^{cA}	13.08 ± 1.20^{dB}	0.555
	100 ppm	24.36 ± 2.22^{aA}	21.23 ± 0.49^{bA}	19.44 ± 0.34^{bcA}	18.23 ± 0.35^{cA}	15.45 ± 0.63^{dA}	
	200 ppm	24.61 ± 0.54^{aA}	21.36 ± 1.39^{bA}	19.45 ± 0.23^{cA}	18.28 ± 0.34^{cdA}	17.16 ± 1.63^{dA}	
	300 ppm	24.83 ± 0.74^{aA}	22.20 ± 0.95^{bA}	19.98 ± 0.93^{cA}	18.55 ± 0.94^{cdA}	17.34 ± 0.29^{dA}	
*b**	Control	14.75 ± 0.50^{aA}	13.32 ± 0.28^{bA}	12.98 ± 0.77^{bcA}	12.26 ± 0.56^{cA}	13.48 ± 0.24^{bA}	0.361
	100 ppm	14.69 ± 1.19^{aA}	13.08 ± 0.11^{bA}	12.53 ± 0.50^{bA}	12.54 ± 0.24^{bA}	12.58 ± 0.74^{bAB}	
	200 ppm	14.68 ± 0.51^{aA}	13.21 ± 0.96^{bA}	12.36 ± 0.15^{bA}	12.37 ± 0.22^{bA}	12.46 ± 0.75^{bAB}	
	300 ppm	14.17 ± 1.36^{aA}	13.39 ± 0.15^{abA}	12.33 ± 0.62^{bcA}	12.32 ± 0.30^{bcA}	11.74 ± 0.26^{dB}	

a–e: Different lowercase letters on the same row indicate that there is a significant difference ($P < 0.05$) between different treatments within same storage day

A-D: Different capital letters in the same column indicate that there is a significant difference ($P < 0.05$) between different storage times within same treatment

LBCWE: Lyophilized black carrot water extract

SEM: Standard Error of Means

(Table 1). The a* values decreased continuously during storage in all ground beef samples with and without extract, while the most decrease was in the control group. The highest difference among control and samples with extract was on the 12th day of storage. On day 12 of storage, the redness values of the control samples were 2.37, 4.08 and 4.26 units lower than those of the groups containing 100, 200 and 300 ppm LBCWE (Table 3, Fig. 1d). On this day, the a* values of the groups containing 200 and 300 ppm extract were higher due to the less formation of metmyoglobin in these groups (Fig. 1c). Similar changes were observed in a study by Zamuz et al. (2018). Of course, the loss of red colour in the ground beef appearance during storage is undesirable. This discoloration was reduced with the addition of extract.

Microbial counts

All variation sources and possible two-way interactions had a significant effect ($P < 0.01$) on microbial counts of ground beef (Table 2). Microbial counts determined during storage in the ground beef with or without LBCWE are given in Tables 2, 4. Total aerobic mesophilic bacteria (TAMB) counts of the control group without extract were higher than the ground beef samples containing LBCWE.

TAMB counts of all treatment groups steadily increased during storage. The greatest increase in the storage period was in the control samples, and lower counts were found in the ground beef groups with extract during storage. The highest logarithmic increase in the count of TAMB was between the 9th and 12th days of storage, there was an increase of 1.44 log units in the control samples and 0.88 log units in the 300 ppm LBCWE group (Table 4). On the 12^{th} day of storage, these counts were determined as 7.42, 6.62, 6.34 and 6.00 log CFU/g in control, 100, 200 and 300 ppm groups, respectively. Considering these results, it is understood that adding 300 ppm extract to ground beef provides an advantage of 1.42 log units in TAMB counts compared to the control during storage at 2 °C for 12 days in HiOx-MAP. The count of TAMB is an important criterion in terms of the consumability of ground beef and 6.0 log CFU/g is maximum acceptable value. The counts of TAMB determined in all of the samples at the end of the 9th day was below 6.00 log CFU/g. On day 12, the TAMB count was determined as 6.0 log CFU/g only in samples with 300 ppm extract. As a result, the shelf life of ground beef was extended by 3 days with MAP + 300 ppm extract application.

On the other hand, the highest psychrotrophic bacteria counts were determined in control samples among treatments groups, and these counts increased during storage. On the 6th, 9th and 12th days of storage, generally samples with LBCWE had lower values than the control (Table 4).

Table 4 The microbiological properties of HiOx-MAP ground beef with different levels of LBCWE during 12 days of storage at 2 ± 0.5 °C (n = 4)

	Extract levels	Storage time (days)					SEM
		1	3	6	9	12	
Total aerobic mesophilic bacteria (log CFU/g)	Control	3.79 ± 0.10^{dB}	4.54 ± 0.07^{cA}	5.89 ± 0.16^{bA}	5.98 ± 0.06^{bA}	7.42 ± 0.46^{aA}	0.123
	100 ppm	3.85 ± 0.15^{dAB}	4.46 ± 0.14^{cA}	4.67 ± 0.06^{cC}	6.02 ± 0.25^{bA}	6.62 ± 0.05^{aB}	
	200 ppm	3.71 ± 0.14^{dB}	4.46 ± 0.39^{cA}	4.53 ± 0.15^{cBC}	5.54 ± 0.16^{bB}	6.34 ± 0.14^{aB}	
	300 ppm	4.27 ± 0.39^{cA}	3.98 ± 0.03^{cB}	4.85 ± 0.05^{bB}	5.12 ± 0.34^{bB}	6.00 ± 0.17^{aB}	
Psychrotrophic bacteria (log CFU/g)	Control	4.13 ± 0.19^{dAB}	4.56 ± 0.08^{cA}	5.92 ± 0.21^{bA}	6.04 ± 0.12^{bA}	6.76 ± 0.21^{aA}	0.092
	100 ppm	4.11 ± 0.08^{cAB}	4.62 ± 0.26^{bA}	4.69 ± 0.02^{bAB}	6.30 ± 0.18^{aA}	6.56 ± 0.19^{aA}	
	200 ppm	3.93 ± 0.08^{dB}	4.43 ± 0.15^{cA}	4.51 ± 0.18^{cC}	5.53 ± 0.18^{bB}	6.16 ± 0.03^{aB}	
	300 ppm	4.33 ± 0.29^{cA}	4.77 ± 0.15^{bA}	4.87 ± 0.07^{bB}	5.04 ± 0.09^{bC}	6.27 ± 0.08^{aB}	
Micrococcus/ Staphylococcus (log CFU/g)	Control	3.62 ± 0.03^{cB}	3.77 ± 0.02^{bcA}	3.80 ± 0.13^{bcA}	3.84 ± 0.06^{bA}	4.23 ± 0.19^{aA}	0.071
	100 ppm	3.64 ± 0.14^{aB}	3.69 ± 0.02^{aAB}	3.56 ± 0.05^{aB}	3.83 ± 0.06^{aA}	3.75 ± 0.40^{aB}	
	200 ppm	3.61 ± 0.10^{aB}	3.62 ± 0.08^{aB}	3.64 ± 0.05^{aB}	3.68 ± 0.04^{aB}	3.75 ± 0.15^{aB}	
	300 ppm	3.96 ± 0.04^{aA}	3.76 ± 0.07^{bA}	3.56 ± 0.06^{dB}	3.63 ± 0.08^{cdB}	3.71 ± 0.06^{bcB}	
Lactic acid bacteria (log CFU/g)	Control	2.97 ± 0.08^{eA}	3.34 ± 0.18^{dA}	4.49 ± 0.06^{cA}	4.77 ± 0.18^{bA}	5.72 ± 0.08^{aA}	0.109
	100 ppm	2.81 ± 0.21^{dAB}	3.39 ± 0.16^{cA}	3.64 ± 0.13^{cB}	4.74 ± 0.27^{bA}	5.40 ± 0.41^{aA}	
	200 ppm	2.71 ± 0.06^{eB}	3.22 ± 0.11^{dA}	3.59 ± 0.12^{cB}	4.55 ± 0.06^{bA}	5.38 ± 0.32^{aA}	
	300 ppm	2.83 ± 0.08^{eAB}	3.19 ± 0.30^{dA}	3.58 ± 0.17^{cB}	4.11 ± 0.11^{bB}	5.46 ± 0.19^{aA}	
Pseudomonas (log CFU/g)	Control	3.92 ± 0.13^{cA}	4.11 ± 0.12^{cA}	5.19 ± 0.07^{bA}	5.21 ± 0.11^{bB}	6.21 ± 0.22^{aA}	0.092
	100 ppm	3.64 ± 0.08^{cBC}	3.95 ± 0.15^{bcAB}	4.16 ± 0.39^{bB}	5.67 ± 0.13^{aA}	6.03 ± 0.15^{aA}	
	200 ppm	3.47 ± 0.06^{cC}	3.55 ± 0.17^{cC}	3.61 ± 0.28^{cC}	4.94 ± 0.08^{bC}	5.34 ± 0.05^{aB}	
	300 ppm	3.74 ± 0.19^{cAB}	3.84 ± 0.06^{cB}	4.43 ± 0.05^{bB}	4.58 ± 0.13^{bD}	4.97 ± 0.08^{aC}	
Enterobacteriacea (log CFU/g)	Control	2.35 ± 0.11^{dA}	3.00 ± 0.13^{bA}	2.80 ± 0.03^{bcA}	2.76 ± 0.12^{cA}	3.69 ± 0.16^{aA}	0.080
	100 ppm	2.18 ± 0.11^{bAB}	2.05 ± 0.06^{bB}	2.18 ± 0.26^{bB}	2.39 ± 0.25^{bB}	2.89 ± 0.16^{aB}	
	200 ppm	2.09 ± 0.15^{cB}	2.13 ± 0.16^{cB}	2.16 ± 0.12^{bcB}	2.37 ± 0.04^{bB}	2.87 ± 0.04^{aB}	
	300 ppm	2.14 ± 0.15^{abAB}	2.07 ± 0.07^{bB}	2.15 ± 0.14^{abB}	2.34 ± 0.08^{aB}	2.23 ± 0.13^{abC}	

a-e: Different lowercase letters on the same row indicate that there is a significant difference ($P < 0.05$) between different treatments within same storage day

A-D: Different capital letters in the same column indicate that there is a significant difference ($P < 0.05$) between different storage times within same treatment

LBCWE: Lyophilized black carrot water extract

SEM: Standard Error of Means

In present study, the highest *Micrococcus/Staphylococcus* (Gram-positive, catalase-positive cocci) counts were determined in control group during storage period (except on day 1), but there were no significant differences among groups with LBCWE at the end of storage (Table 4).

Regarding lactic acid bacteria (LAB), the highest counts were determined in the control group, while the lowest values were generally found in the 300 ppm group (Tables 2, 4). The LAB counts of all treatment groups increased continuously during storage, and the highest difference between the control group and samples with LBCWE was observed on day 6 of storage. The most suitable pH degrees for the growth of LAB are 5.5–5.8. Therefore, there was more growth in the control samples compared to the samples with extract. Also, LAB are facultative anaerobic and have the ability to grow in high CO_2 environment. Therefore, they can form the dominant flora in modified atmosphere packaged meats. In a study examining the effects of different packaging methods on the quality of ground beef patties were studied, it was found that the counts of LAB increased by 7–8 log units during storage at 0–2 °C for 20 days in 80% O_2 + 20% CO_2 and LAB formed the dominant flora (Rogers et al. 2014). Also, Degirmencioglu et al. (2012) determined that the counts of LAB in modified atmosphere packed (70% O_2 + 30% CO_2) ground beef stored at 4 °C for 7 days ranged from 2.47 to 5.03 log CFU/g.

The growth of *Pseudomonas* was inhibited by LBCWE. The count of *Pseudomonas* reached the maximum count on the 12th day of storage. On this day of storage, a difference of 1.24 log units occurred between the control and 300 ppm groups (Table 4). This difference is important for delaying the spoilage. Yim et al. (2019) detected similar *Pseudomonas* counts in beef packaged in 80% O_2 + 20% CO_2 and stored at 4 ± 2 °C for 15 days.

In this research, *Enterobacteriaceae* was suppressed during storage by adding LBCWE to ground beef. Although the *Enterobacteriaceae* counts generally increased in all treatment groups during storage, the highest counts were determined in the control samples. On the 12th day of storage, the counts detected in ground beef samples with 300 ppm extract were 1.46 log CFU/g lower than those found in the control group (Table 4). The antimicrobial properties of plant extracts are largely due to the hydroxyl groups of the phenolic component, which its react with the cell membrane and disrupt its structure. In a study conducted by Langrooti et al. (2018), *Enterobacteriaceae* increased (from 3.74 to 7.64 log CFU/g) continuously in control beef steaks packaged in 80% O_2 + 20% CO_2 and stored at 4 ± 1 °C for 20 days.

Conclusion

The results of present study showed that lyophilized black carrot water extract (LBCWE) had antimicrobial, food colorant and antioxidant properties due to its anthocyanin and phenolic compounds. It was determined that the extract had strong antimicrobial activity in the preservation of ground beef in HiOx-MAP. Also, the effect of the extract on the myoglobin oxidation was more than its effect on lipid oxidation in the HiOx-MAP ground meat. LBCWE increasingly delayed the discoloration and the formation of metmyoglobin in HiOx-MAP ground meat compared to control samples. Thus, the highest a* values were determined in ground beef samples with 300 ppm LBCWE during storage. Inhibition of *Pseudomonas* and *Enterobacteriaceae* in the HiOx-MAP ground beef could be achieved using 300 ppm LBCWE. Considering that the maximum acceptable total aerobic mesophilic bacteria count for ground beef is 6.0 log CFU/g, the shelf life of ground beef was extended by 3 days with HiOx-MAP + 300 ppm extract application. The addition of 300 ppm LBCWE reduced the negative effects of HiOx-MAP on lipid oxidation in ground meat during storage. However, studies can be conducted on the use of higher levels of LBCWE extracts to further extend the shelf-life by preventing microbial growth and lipid oxidation in ground meat with HiOx-MAP.

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Declarations

Conflict of interest The authors declare no potential conflict of interest.

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