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Properties of black carrot extract and its efficacy for improving the storage quality of vacuum packaged fresh meat products

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Abstract

Natural plant extracts have been widely used in the food industry in recent years as natural colourants, antioxidants and antimicrobials due to consumer health concerns. In this study, the effects of different levels of black carrot water extract (BCWE) (control without BCWE, 100, 200 and 300 ppm) on metmyoglobin (MetMb), lipid oxidation, colour and microbial counts of vacuum-packed ground beef were evaluated during chilled storage for 15 days. MetMb, pH, lipid oxidation, redness (a^*) values and microbial counts of ground beef were significantly ($p < 0.01$) affected by BCWE treatment. During storage, the lowest lipid oxidation values were determined in the BCWE groups ($p < 0.05$). BCWE groups had higher a^* values and lower MetMb content at the end of storage than control. BCWE contributed to reduce the growth of *Enterobacteriaceae*. The 200-ppm BCWE group had approximately 2 log units lower *Enterobacteriaceae* counts at the end of storage compared to the control. These results showed that the anthocyanin-rich BCWE is a promising natural additive to overcome the problems in vacuum-packed ground meat. Consequently, 200-ppm BCWE can be recommended for use in vacuum-packed ground beef to prevent discolouration, lipid oxidation and microbial growth during chilled storage.

KEYWORDS
black carrot extract, discolouration, ground beef, microbial quality, oxidative stability

1 | INTRODUCTION

Black carrots (*Daucus carota* L.) are a 2-year-old cultivated vegetable, belonging to the Umbelliferae family. They have been cultivated for 3000 years in the Middle and Far East and Turkey, where are linked for its place of origin.[1] Black carrots contain phenolic compounds, anthocyanins, and vitamins C and E, which significantly increase their antioxidant capacity.[2] Due to the mono-acylated structure of anthocyanins, the colour of black carrot is preserved at the natural pH of foods and contributes to antioxidant capacity.[3] Black carrot also has antimicrobial properties against microorganisms such as *Listeria monocytogenes* and *Salmonella* Typhimurium.[4]

Fresh meat products such as ground beef are suitable for all types of spoilage due to their structural features (composition, pH and a_w) and preparation technologies such as mincing and mixing. These products provide an ideal growth environment for many microorganisms that reduce the durability of the product and pose a potential risk for consumer health.[5,6] Lipid oxidation, microbial growth and discolouration are the main factors affecting the deterioration of meat and meat products. Discolouration is due to the loss of the ability to reduce MetMb to OxyMb and is closely related to oxidative denaturation of meat pigments.[7,8]

In addition, due to the formation of MetMb, which occurs at different levels depending on the characteristics of the product and the packaging, the colour of the meat varies considerably and causes the attractiveness of the product to disappear in a short time. Vacuum and modified atmosphere packaging are generally applied for preservation of ground meat, but the application of vacuum packaging is limited due to disadvantages such as sour/cheesy smell caused by the growth of anaerobic bacteria and colour appearance not preferred by

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consumers.[9,10] These disadvantages related to ground meat reveal that additional technological processes are needed to maintain the quality of ground meat. Considering the potential toxic or carcinogenic effects of synthetic antioxidants and colourants,[11,12] it is recommended to add natural antioxidants and colourants to maintain the quality of ground meat.[5,13,14]

A previous study found that lipid oxidation, discolouration, and microbial deterioration were reduced by the addition of black carrot extract to ground beef packaged under modified atmosphere conditions (MAP; 80% O_2 + 20% CO_2), and the shelf life was extended by the combination of MAP + 300 ppm extract compared with MAP-control without extract.[15] However, there has been no published study examining the effects of black carrot extract on vacuum-packed ground beef. Therefore, the objectives of this study were to preserve the quality and extend the shelf life of ground beef prepared for consumption by adding different amounts of black carrot extract and by reducing the negative effects of vacuum packaging.

2 | MATERIALS AND METHODS

2.1 | Materials

Beef top round (mainly consisting of the semimembranosus muscle) of the cross Brown Swiss were obtained from Meat and Milk Board Slaughterhouse (Erzurum/Turkey) after 24-h postmortem. Black carrot (*D. carota* L.) was obtained from a local market in Erzurum/Turkey. Packaging material from a private company (Südpack Verpackungen GmbH & Co, Ochsenhausen, Germany) was used in the research.

2.2 | Methods

2.2.1 | Plant material and processing

Black carrot water extract (BCWE) was prepared based on the method described by Alp and Aksu.[5] Black carrots were washed, peeled, sliced and smashed thoroughly in a kitchen-type blender. Then, 400 ml of distilled water was added to 20 g of smashed black carrots, and the mixture was homogenized using an ultraturrax (IKA T25 Werk, Germany) at 13,500 rpm for 30 s. After homogenization, the homogenate was stirred at room temperature for 15 min on a magnetic stirrer and filtered through Whatman paper (N. 1). A part of the water in the filtrate was evaporated for 60 min at 40°C using a rotary evaporator. The concentrated mixture was frozen at −38°C and lyophilized at −50°C. The obtained lyophilized powders were kept in refrigerator conditions until used.

2.2.2 | Preparation of vacuum packaged ground beef and addition of the BCWE

In this trial, muscles were obtained from two beef carcasses (for Replication 1 and Replication 2) after 24-h postmortem. Under hygienic conditions, they were ground twice through a meat grinder with a 3-mm diameter plate and kept at 4°C until used (approximately 1 h). Forty-eight lots (about 200 g each) of ground beef were obtained from a carcass and randomly divided into four treatment groups. Four treatments were prepared as follows: control (C; without BCWE) group, and ground beef samples treated with 100-ppm BCWE, 200-ppm BCWE, and 300-ppm BCWE. The extracts were dissolved in 2 ml of sterilized water and added homogeneously to the ground beef. To ensure equality, 2 ml of sterilized water was added to the control ground beef samples. Then, each ground beef sample was mixed by hand with gloves. Considering the storage period (1, 3, 6, 9, 12, and 15 days), a total of 96 ground beef samples were prepared for two replicates, 12 for each treatment, and all treatments were placed in the packaging material and vacuum-packaged. A Multivac vacuum packing unit (Germany, Multivac 300/16 Sepp. Haggenmuller D 87787 Wolgertschwenden) was used for vacuum packaging of the samples, and the properties of the packaging material were as follows; (PA/PE [polyamide/polyethylene], 3 seal-bag GB 65; thickness 65 μm; oxygen transmission rate [OTR]: ≤50 cm^3/m^2/day. Bar at 23°C, 50% RH; CO_2 transmission rate [COTR]: ≤200 cm^3/m^2/day. Bar at 23°C, 0% RH; N_2 transmission rate [NTR]: ≤15 cm^3/m^2/day. Bar at 23°C, 0% RH and water vapor transmission rate [WVTR]: ≤9 g/m^2/day 38°C, 90% RH). Vacuum-packed ground beef samples were stored at 2 ± 0.5°C for 15 days and analysed on the 1st, 3rd, 6th, 9th, 12th and 15th days of storage.

2.2.3 | Characteristic properties of BCWE

Metal chelation ratio analyses were performed according to Dinis et al.,[16] and the results were calculated as equivalent to standard antioxidants (BHA, BHT and tocopherol). Titration acidity and pH were measured by the method described by Cemeroğlu.[17] DPPH-radical scavenging activity was analysed according to the procedures described by Blois.[18] Antioxidant activity results were calculated as trolox equivalent (TE) and IC50 value. Total monomeric anthocyanin analysis was performed according to the pH differential method.[19] Total phenolic content of BCWE were determined as described Singleton et al.[20] and expressed as the gallic acid equivalent (GAE) calculated with the standard solution prepared daily.

2.2.4 | Vacuum packaged ground beef analysis

Thiobarbituric acid reactive substances

According to method described by Aksu,[21] 12 ml of trichloroacetic acid (TCA) solution including 7.5% TCA, 0.1% propyl gallate and 0.1% ethylenediaminetetraacetic acid were added on 2-g meat taken from each sample and homogenized for 30 s using an ultraturrax. The mixture was filtered using Whatman No:1. Then, 1 ml of the 0.02 M thiobarbituric acid (TBA) solution was added to 1 ml of the filtrate. It was kept in a boiling water bath for 40 min, then cooled and centrifuged for 5 min at 2.000 × *g*. Absorbance measurements were

performed at 532 nm (Shimadzu, UV 160 A, Kyoto, Japan). The tetraethoxypropane was used in the preparation of the standard curve, and thiobarbituric acid reactive substances (TBARS) values of samples were calculated as mg malondialdehyde (MDA) per kilogramme meat.[22] The method modified by Aksu and Turan[15] was followed to avoid misinterpretation of the analysis due to the colour resulting from the extract. Accordingly, aliquots of the filtrate obtained as a result of filtration after homogenization with an ultraturrax were transferred to two separate test tubes and mixtures with and without TBA solution were prepared. After these mixtures were kept in a boiling water bath for 40 min, their absorbance values were measured separately. The difference of these measurements was used to calculate the MDA content.

Metmyoglobin

The metmyoglobin (MetMb) percentage of the total myoglobin perceptible was measured by the method described by Kannan et al.[23] A 25-ml ice-cold phosphate buffer (pH 6.8, 40 mM, 4°C) on the 5-g ground beef samples were added, and homogenized using an ultraturrax for 10 s at 13,500 rpm. It was kept in 4°C for 60 min and centrifuged at 3500×*g* for 30 min at 4°C and the supernatant was filtered with filter paper (Whatman No:1). The absorbance of the filtrate was read at 700 nm, 572 nm, and 525 nm by scanning the visible spectrum with a spectrophotometer (Shimadzu, UV 160 A, Kyoto, Japan) and the results were calculated as the percentage of metmyoglobin (MetMb%).

Colour

The colour of ground beef was measured using a portable colorimeter (Minolta CR-400, Osaka, Japan) with an 11-mm diameter measuring area. Before each measurement, the colorimeter was calibrated using a calibration plate of instrument to be $L^* = 97.4$, $a^* = 0.3$, $b^* = 1.7$. The CIE Illuminant C (2° observer angle) was used as the light source in colour measurement of ground meat and extract samples. Nine measurements were taken from each sample for each day of the storage period.

pH

The pH values were determined using a pH meter (Orion 3 Star, Thermo Scientific, Singapore). The pH meter was calibrated with buffer solutions (pH 4.0 and pH 7.0) at 20°C before each measurement. Ten grams of ground beef samples were homogenized with 100 ml of distilled water for 60 s using an ultraturrax. Then, the homogenate was used for pH analysis.

Microbiological analysis

For each day of storage time, 25 g of sample from each ground beef was prepared individually by homogenizing with 225 ml of saline solution containing 0.85% NaCl in a Stomacher (Laboratory Blender-400, Seward Medical, London, UK) for 1 min. Then, serial dilutions were prepared and the surface plate method was used for enumeration. Total aerobic mesophilic bacteria and psychrotrophic bacteria were incubated aerobically on Plate Count Agar (Oxoid CMO325, Hampshire, UK) at 30°C for 2 days and 10°C for 7 days, respectively. Mannitol Salt Phenol-red Agar (Merck, Darmstadt, Germany) was used for *Micrococcus/Staphylococcus* (*M/S*) bacteria, and plates were aerobically incubated at 30°C for 48 h. *M/S* counts were determined taking into account the catalase positive cocci. For *Pseudomonas*, *Pseudomonas* Agar Base (Oxoid CMO559, Hampshire, UK) with Cetrimid-Fusidin-Cephaloridin (CFC) Selective Agar Supplement (Oxoid SR0103, Hampshire, UK) was used and plates were aerobically incubated at 25°C for 48 h. *Pseudomonas* counts were determined taking into account the oxidase (+) colonies. *Enterobacteriaceae* and lactic acid bacteria (LAB) were incubated anaerobically (Anaerocult A, Merck) on Violet Red Bile Glucose Agar (Oxoid CMO485, Hampshire, UK) and De Man Rogosa Sharpe agar (Oxoid CMO361, Hampshire, UK) at 30°C for 2 days, respectively. LAB counts were determined taking into account the catalase (−) colonies. Counts were expressed as colony-forming units per gram of sample (log CFU/g).

2.2.5 | Statistical analysis

A completely random block design was used in the statistical analysis. The model included treatments (Control, 100-ppm BCWE, 200-ppm BCWE and 300-ppm BCWE) and storage time (1, 3, 6, 9, 12 and 15 days) as main effects and all their interactions. Experiments were replicated twice for each block design as completely random. Each muscle from each carcass served as a block and ground meat from each carcass was assigned randomly for treatment. The results were analysed by SPSS 22.0 statistical package, comparison of means were made using the 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.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of BCWE

Bioactive compound content, physicochemical and antioxidant properties of BCWE are given in Table 1. The total phenolic and total monomeric anthocyanin contents in BCWE were determined as 2788.89-mg GAE/100 g and 1219.02-mg cyanidine-3-glucoside (C3G)/100 g, respectively. Our findings were consistent with the values reported by Aksu and Turan[15] for black carrot extract. Studies on black carrots reveal that the total anthocyanin content is in a very wide range. Montilla et al.[1] and Algarra et al.[2] reported that the amounts of total anthocyanin in the fresh tissue were 1.5–17.7 mg/100 g and 93.8–126.4 mg/100 g, respectively. Kammerer et al.[24] also found that the anthocyanin amounts of different varieties of black carrot were between 45.4 and 17.4 g/kg dry weight. The anthocyanin contents found in the current study are quite high. This can be attributed to the high solubility of anthocyanins in water,[25] and the better preservation of anthocyanins by lyophilization compared with other drying methods.[26] On the other hand, the pH and

acidity values of the extracts obtained from fruits and vegetables are important for the colour pigments. Anthocyanins in the composition of the extracts show colour properties ranging from red to blue according to the pH of the medium.[3] In the present study, the average pH and titratable acidity of BCWE were 5.90 and 0.29%, respectively. The *L** value expressing the darkness/lightness was 18.60 and the *a** value indicating redness was 2.52. Black carrot anthocyanins show structural colour changes depending on the flavylium cation (red colour) under acidic conditions, and the quinoidal anhydrobase (purple) below pH 7.[27] Accordingly, BCWE had a purplish colour at its natural pH. Moreover, the stability of black carrot anthocyanins is better maintained under acidic conditions (pH <5).[3] Similarly, Aksu et al.[28] determined that anthocyanins in purplish-coloured red cabbage extract provide a redder and more stable colour below pH 5.0 in a food adjusted to different pH values.

The use of anthocyanins as natural colourants as an alternative to synthetic colourants in food industry is increasing day by day. Anthocyanins not only give attractive colour properties to foods, but also increase the oxidative stability of the food due to their high antiradical capacities.[29] Regarding the antioxidant activity of BCWE, DPPH radical scavenging activity was found to be 45.17 mmol TE/100 g and IC50 (μg) value was 228.03. The metal chelating activity values were 83.38 mg BHA/100 g, 158.84 mg BHT/100 g and 28.23 mg tocopherol/100 g, respectively. Accordingly, BCWE has an important radical scavenging, high reactive hydroxyl and superoxide anion radical removal properties. Algarra et al.[2] determined that total phenolic amount and DPPH radical scavenging activity of fresh tissue of black carrot were 187.8–492 mg GAE/100 g and 17.6–240 μM TE/100 g, respectively. The results of the present study showed that anthocyanin-rich BCWE can be used as a natural colourant and antioxidant instead of synthetic additives in the food industry.

TABLE 1 Bioactive compound content, physicochemical and antioxidant properties of black carrot water extract (BCWE)

Properties		Mean ± Std*
pH		5.90 ± 0.02
Titratable acidity (% citric acid)		0.29 ± 0.03
*L** colour value		18.60 ± 0.67
*a** colour value		2.52 ± 0.38
*b** colour value		−0.76 ± 0.13
Total phenolic content[a]		2788.89 ± 39.76
Total anthocyanin content[b]		1219.02 ± 16.70
Metal chelating activity	(mg BHA/100 g)	83.38 ± 1.79
	(mg BHT/100 g)	158.84 ± 5.68
	(mg tocopherol/100 g)	28.23 ± 0.81
DPPH radical scavenging activity	(mmol TE/100 g)	45.17 ± 1.68
	IC50 (μg)	228.03 ± 1.65

Abbreviations: Std*, standard deviations; TE, trolox equivalent; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; IC50, concentration required to scavenge 50% of the initial DPPH radical.
[a]Results were expressed as mg gallic acid equivalent (GAE)/100 g.
[b]Results were expressed as mg cyanidine-3-glucoside (C3G)/100 g.

3.2 | Ground beef

3.2.1 | pH values

As shown in Table 2, BCWE treatment, storage time, and two-way interaction of these parameters significantly ($p < 0.01$) affected the pH values of vacuum-packed ground meat. The addition of BCWE reduced pH values compared with control, and this reduction was dependent on the extract concentration ($P < 0.05$). Increasing the amount of BCWE added to ground beef result in a gradual decrease in pH values (Figure 1A). This gradual decrease in pH is probably due to the acidity of the extract (Table 1). The BCWE-containing samples had the lowest ($p < 0.05$) pH values during storage compared to the control, especially the groups with 200- and 300-ppm extract. The pH values fluctuated during storage, and the pH of all groups generally decreased after Day 9. This effect is important for extending the shelf life of ground meat because bacterial growth, lipid and protein oxidation are slower at low pH. Under low pH conditions, the lipid oxidation rate may decrease as protons are easier to transfer to the alkyl, alkoxy, and/or peroxyl lipid radicals formed during autoxidation.[30] Consistent with our findings, Turan and Şimşek[14] reported that the pH values of beef patties treated with black mulberry extract were lower than the control during 15 days of chilled storage in both aerobic and vacuum conditions. Similarly, a dose-dependent decrease in

TABLE 2 Effects of experimental factors and their interactions on pH, TBARS, MetMb, colour values, and microbial counts of vacuum-packed ground beef treated with different levels of BCWE

				Colour values			Microbial counts					
	pH	TBARS	MetMb	L*	a*	b*	TAMB	TAPB	Pseudomonas	LAB	*M/S*	*Enterobacteriaceae*
Treatment (*T*)	**	**	**	NS	**	NS	**	**	**	**	**	**
Storage time (*ST*)	**	**	**	**	**	**	**	**	**	**	**	**
T × *ST*	**	**	**	**	**	**	**	**	*	**	**	**

Abbreviations: BCWE, black carrot water extract; LAB, lactic acid bacteria; *M/S*, *Micrococcus/Staphylococcus*; NS, not significant; TAMB, total aerobic mesophilic bacteria; TAPB, total aerobic psychrotrophic bacteria; TBARS, thiobarbituric acid reactive substances; MetMb, metmyoglobin (%); *L**, Lightness; *a**, redness; *b**, yellowness.
*$p < 0.05$. **$p < 0.01$.

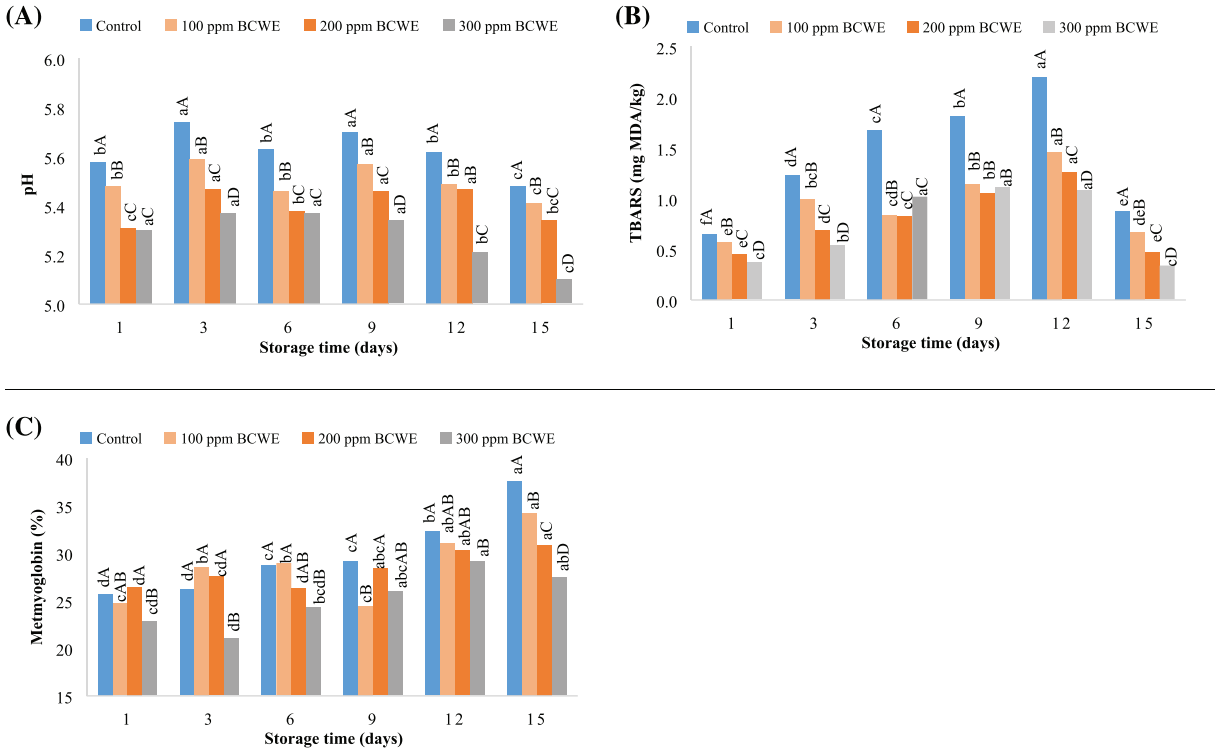

FIGURE 1 Effects of different levels of black carrot water extract on (A) pH, (B) TBARS and (C) metmyoglobin values of vacuum-packed ground beef during chilled storage. Different lowercase letters (a–f) on the same bar chart represent significant differences between storage days for the same treatment ($p < 0.05$). Different capital letters (A–D) in the same bar chart represent significant differences between treatments on the same storage day ($p < 0.05$)

pH values of fresh meat products incorporated with various vegetable extracts has been reported in previous studies.[31–33]

3.2.2 | Thiobarbituric acid reactive substances

A significant ($P < 0.01$) interaction effect of BCWE treatment and storage time was observed for TBARS values expressing the degree of lipid oxidation (Table 2). Figure 1B presents the antioxidant potential of BCWE on lipid oxidation of vacuum-packaged ground beef samples during 15 days chilled storage. Overall, TBARS values of the control and BCWE-added samples increased until Day 12 and then decreased significantly ($P < 0.05$) on Day 15. The present findings are consistent with some researchers who reported that TBARS formation increased to a maximum and then decreased over storage.[34] This decline trend in TBARS can be attributed to the decomposition of MDA into other organic by-products that do not react with thiobarbituric acid, such as alcohols and acids. Low molecular weight volatile oxidation products may be lost during prolonged storage. It can also be related with degradation by bacteria such as *Pseudomonas* and *Enterocacteriacea*, which selectively attack and use carbonyl compounds such as MDA.[35] As seen in our microbiological results (Table 4), *Pseudomonas* and *Enterobacteriaceae* counts of ground beef samples reached their highest level in the end of the storage period, where TBARS values decreased. The loss/disappearance rate in MDA content during storage through intermolecular reactions may have exceeded the rate of MDA produced by lipid oxidation.[36] Moreover, secondary lipid oxidation products (aldehydes, ketones, epoxydes and carbonyl compounds including MDA) can react with peptides, proteins, free amino groups, phospholipids and nucleic acids.[36,37] Because only free MDA is measured in the TBARS assay, MDA-protein interaction can lead to lower TBARS values.[35]

During the storage period, the highest TBARS values were determined in the control samples (0.65–2.20 mg MDA/kg), while the lowest values were in the 300-ppm BCWE-added ones (0.37–1.11 mg MDA/kg). Regarding the TBARS contents of the control, a maximum value of 2.20-mg MDA/kg was obtained on the 12th day of chilled storage. On the other hand, all samples treated with BCWE were exposed to less oxidation than the control group during storage ($P < 0.05$). The MDA contents of the groups treated with 100, 200 and 300 ppm BCWE on Day 15 of storage were 23.86%, 46.59% and 61.36% lower, respectively, compared with the control without extract ($p < 0.05$). According to the present results, the use of 300 ppm BCWE was more effective than 200- and 100-ppm levels in protecting ground beef from lipid oxidation. Campo et al.[38] proposed that 2-mg MDA/kg could be accepted as the limiting threshold for rancidity in terms of the sensory acceptability of lipid oxidation in beef. Accordingly, the control samples were unacceptable due to exceeded threshold after 12 days of storage, while the TBARS values of all samples containing BCWE were below the acceptable limit values until the end of the storage period.

These results demonstrate that BCWE has a potential antioxidant effect against lipid oxidation in ground beef. The protective effect against lipid oxidation can be attributed to the fact that BCWE contains various compounds with high antioxidant capacity, especially anthocyanins. In addition to their use as colourants, anthocyanins can also be nutritionally attractive because they are considered to be effective scavengers of free radicals and have bioavailable antioxidant properties.[24,39] Many studies in the literature have shown that black or purple carrot has high antioxidant capacity.[2,3,15] Considering that BCWE has high phenolic content and antioxidant capacity, it can be used as a natural antioxidant to control lipid oxidation in ground beef instead of synthetic antioxidants. The results obtained in the present study are similar to many studies showing that natural antioxidants such as plants, fruits and vegetables delay the oxidative deterioration in fresh meat and meat products.[5–8,13–15,31–33,40] In this regard, Ekici et al.[41] demonstrated that black carrot concentrate (BCC) increased the antiradical activities and total phenolic content of Turkish dry-fermented sausage samples. They reported that the antiradical activity of the samples incorporated with 2% BCC was twice that of the sample containing carmine.

3.2.3 | Metmyoglobin (MetMb)

The treatment, storage time and interaction of treatment × storage time had a very significant effect ($p < 0.01$) on MetMb content of ground beef samples (Table 2). As seen in Figure 1C, the MetMb content of the samples generally increased as the storage time progressed ($p < 0.05$). However, the increase in MetMb percents of the BCWE groups during storage was lower than in the control group without extract. In this context, the MetMb content of the 300-ppm BCWE group was lower than the control on all days except Day 9. In addition, the MetMb levels of the 100- and 200-ppm extract groups were similar to the control group until Days 6 and 12, respectively ($P > 0.05$), but on Day 15, all BCWE groups had lower MetMb levels than the control ($p < 0.05$). The lowest MetMb contents during storage were generally in the group containing 300-ppm BCWE ($p < 0.05$). At the end of storage, MetMb content increased by 46.23% in the control compared with Day 1, while this increase ranged from 16.62% to 38.51% in the BCWE-containing groups. In other words, the increase in MetMb contents of vacuum-packed ground beef samples containing BCWE was 7.72%–29.61% lower compared with the control group, considering the values on Days 1 and 15 (Figure 1C). Greene et al.[42] reported that meats with 40% metmyoglobin content are not preferred considering consumer preferences. All ground beef samples in the current study maintained the acceptable level for MetMb after 15 days of storage, and this was dependent on the increase in BCWE concentration. Previous studies have revealed that pigment oxidation and lipid oxidation are closely related.[14,15] Although vacuum packaging minimizes the contact of meat with oxygen, it can cause a certain degree of oxidation due to residual oxygen inside the bag and oxygen leaking from the outside through the packaging film.[43] As expected, vacuum packaging prevents the formation of MetMb by limiting the exposure to oxygen, but this study revealed that vacuum packaging + BCWE treatment was more effective in delaying meat discolouration. On the 15th day of storage, there were remarkable differences of about 3%, 7% and 10% between the MetMb contents of the control sample and the 100-, 200- and 300-ppm BCWE groups, respectively ($p < 0.05$). The prevention of discolouration by BCWE can be attributed to its metal chelating and radical scavenging capability, thereby limiting the oxidation of oxymoglobin to MetMb. Therefore, the addition of BCWE to vacuum-packed ground beef may be recommended to delay pigment oxidation and contribute to shelf life extension. Similarly, Turan and Şimşek[14] found that beef patties treated with lyophilized black mulberry (*Morus nigra* L.) water extract had lower metmyoglobin values than control groups during storage.

3.2.4 | Colour values

The addition of BCWE had no effect on lightness (L^*) and yellowness (b^*) values of ground beef samples ($p > 0.05$). However, the significant ($p < 0.01$) effects of BCWE treatment, storage time and treatment × storage time interaction were observed on the redness (a^*) values (Table 2). The changes in colour (L^*, a^* and b^*) values of vacuum-packed ground beef samples with and without BCWE during chilled storage are presented in Table 3. A decrease in lightness as a function of increasing BCWE concentration was observed on Day 1 ($p < 0.05$), but there were no differences ($p > 0.05$) in L^* values for all treatments from Day 3. Generally, an increase in L^* values was observed from Day 6, but only that observed in the 200- and 300-ppm BCWE groups from Day 9 to Day 12 was significant ($p < 0.05$). On the other hand, the inclusion of BCWE in ground beef contributed to the improvement of redness. There was no significant difference between all treatments in redness values on Day 1 ($p > 0.05$). However, the a^* values were higher in BCWE-added groups at the end of storage ($p < 0.05$, Table 3). Higher a^* values were detected in samples with high extract concentration until Day 12, but especially the 100-ppm BCWE group had the highest redness on Days 12 and 15. The lowest a^* colour values were on Days 6 and 9 in all treatments, while an increase in redness was observed on the following storage days ($p < 0.05$).

The colour values (especially the a^* value) of fresh meat and meat products are associated with myoglobin, oxymyoglobin and metmyoglobin. Due to the increasing oxidation of myoglobin during the storage period in vacuum and/or modified atmosphere packaged products, the typical colour of fresh meat disappears and the meat loses its appealing colour. Meat colour is affected by many factors such as pH, oxygen, muscle source, light, packaging method, storage temperature, antioxidants, microbial growth, oxygen consumption rate, mitochondrial respiration, oxygen partial pressure and metmyoglobin reducing activity.[44,45] During storage, the mitochondrial oxygen consumption rate decreases, and the oxymyoglobin layer formed upon exposure to oxygen penetrates deeply into the meat, imparting it an attractive bright red appearance.[46,47] In general, lower

TABLE 3 Instrumental colour values of vacuum-packed ground beef with different levels of BCWE during 15 days of chilled storage

	Treatment (*T*)	Storage time (days, *ST*)						SEM
		1	3	6	9	12	15	*T* × *ST*
*L**	Control	49.42 ± 1.23^{aA}	46.77 ± 0.86^{aA}	47.18 ± 0.80^{aA}	48.65 ± 2.57^{aA}	49.08 ± 2.02^{aA}	48.87 ± 2.56^{aA}	0.754
	100 ppm BCWE	48.30 ± 0.13^{aAB}	48.49 ± 1.30^{aA}	46.98 ± 1.09^{aA}	48.22 ± 2.98^{aA}	48.34 ± 0.50^{aA}	49.78 ± 0.81^{aA}	
	200 ppm BCWE	47.74 ± 0.35^{bcB}	48.71 ± 1.35^{bA}	46.53 ± 0.57^{cA}	47.04 ± 0.62^{cA}	50.46 ± 0.41^{aA}	50.91 ± 0.14^{aA}	
	300 ppm BCWE	44.39 ± 0.25^{cC}	48.64 ± 1.60^{bA}	47.74 ± 0.66^{bA}	48.51 ± 0.45^{bA}	50.48 ± 1.40^{aA}	51.04 ± 0.18^{aA}	
*a**	Control	18.41 ± 0.37^{bA}	16.74 ± 0.91^{cB}	13.29 ± 0.42^{dC}	13.20 ± 0.40^{dB}	18.61 ± 0.42^{aB}	18.27 ± 1.62^{bC}	0.583
	100 ppm BCWE	18.69 ± 0.07^{bA}	16.87 ± 1.17^{cB}	15.22 ± 0.74^{dB}	13.49 ± 0.82^{eB}	20.47 ± 0.52^{aA}	20.27 ± 0.35^{aA}	
	200 ppm BCWE	18.92 ± 0.56^{aA}	20.17 ± 0.77^{aA}	15.63 ± 0.60^{bAB}	13.62 ± 0.97^{cB}	18.86 ± 0.67^{aB}	19.98 ± 0.86^{aB}	
	300 ppm BCWE	19.02 ± 1.03^{abcA}	20.99 ± 1.11^{aA}	16.78 ± 0.70^{cA}	17.11 ± 0.19^{bcA}	18.42 ± 1.29^{abcB}	19.99 ± 3.09^{abB}	
*b**	Control	12.08 ± 0.14^{bAB}	11.77 ± 0.75^{bA}	12.12 ± 0.72^{bA}	12.73 ± 0.36^{abA}	13.43 ± 0.78^{aA}	12.15 ± 0.30^{bBC}	0.439
	100 ppm BCWE	13.05 ± 0.55^{aA}	12.33 ± 0.52^{abA}	11.49 ± 0.33^{bA}	12.09 ± 0.57^{abAB}	11.92 ± 1.16^{abA}	11.67 ± 0.26^{bC}	
	200 ppm BCWE	10.21 ± 1.86^{cB}	12.33 ± 0.28^{abA}	11.45 ± 0.22^{bcA}	11.31 ± 0.88^{bcBC}	11.96 ± 0.83^{abcA}	13.37 ± 0.46^{aAB}	
	300 ppm BCWE	10.63 ± 0.98^{cB}	12.63 ± 0.22^{abA}	11.37 ± 0.55^{bcA}	10.74 ± 0.09^{cC}	13.63 ± 1.19^{aA}	13.90 ± 1.24^{aA}	

Note: Different lowercase letters (a–e) on the same row represent significant differences between storage days for the same treatment ($p < 0.05$). Different capital letters (A–C) in the same column represent significant differences between treatments on the same storage day ($p < 0.05$).
Abbreviations: BCWE, Black carrot water extract; NS, not significant; SEM, standard error of means.

oxygen consumption rate increases oxygen penetration into muscle and colour is more stable.[44] High pH promotes mitochondrial respiration, and deoxygenation of myoglobin causes dark colour. In addition, high pH increases the water retention of the muscle fibres, resulting in swelling of the muscle fibres and less space between them. In this case, because there is less free water, the light cannot be reflected and the light penetrates deep into the tissue, resulting in a dark surface colour.[45,47] An opposite trend is observed at low pH. Accordingly, the decrease in pH values at the end of storage in the present study may be responsible for the increase in lightness and redness by affecting the oxygen consumption rate. On the other hand, the decrease in pH may have increased the redness values by causing a colour change in anthocyanins.

In the present study, the colour values of the extract-added samples were more stable during storage. This can be attributed to the reduction of MetMb formation by BCWE and its high anthocyanin content, and colour properties. Similarly, a previous study by Sadeghinejad et al.[8] reported that the redness values of raw beef patties treated with lyophilized pistachio green hull water extract (LPGH) increased, and the redness values of LPGH-treated patties were better preserved during 8 days of storage, while the lightness was not significantly affected. Black carrot anthocyanins have higher colour stability than anthocyanin compounds in other plants due to the presence of acylated groups. Also, the degradation of black carrot anthocyanins is lower at low temperatures compared with high temperatures, and it is more stable against pH changes.[48] This provides an advantage for ground meat stored at low temperatures such as 0–4°C. Another factor affecting *a** colour value is the pH value of samples. In this study, the lower pH of extract-added ground beef samples compared with the control group (Figure 1A) increased the effect of colour pigments in BCWE. Thus, the redness (*a**) values of the ground beef samples treated with the extract were higher. According to these results, BCWE has promising potential to improve and maintain colour values in vacuum-packed ground meat.

3.2.5 | Microbial counts

As shown in Table 2, the two-way interaction between the treatment and storage time had a very significant effect ($p < 0.01$) on total aerobic mesophilic bacteria (TAMB), total aerobic psychrotrophic bacteria (TAPB), LAB, *Micrococcus/Staphylcoccus* and *Enterobacteriaceae* counts of vacuum-packed ground beef samples, while the effect on *Pseudomonas* counts was significant ($p < 0.05$).

The microbial counts of vacuum-packed ground beef with different levels of BCWE during 15 days of chilled storage are presented in Table 4. The initial (Day 1) TAMB counts ranging from 3.28 to 3.30 log CFU/g were similar to levels reported for vacuum-packed minced meat

TABLE 4 The microbial counts of vacuum-packed ground beef with different levels of black carrot water extract (BCWE) during 15 days of chilled storage

		Storage time (days, *ST*)						
	Treatment (*T*)	**1**	**3**	**6**	**9**	**12**	**15**	**SEM *T* × *ST***
TAMB	Control	3.30 ± 0.04^{eA}	4.69 ± 0.09^{dB}	5.77 ± 0.09^{cA}	6.37 ± 0.26^{bA}	6.90 ± 0.06^{aA}	7.08 ± 0.09^{aA}	0.081
	100 ppm BCWE	3.28 ± 0.02^{eA}	5.06 ± 0.10^{dA}	5.72 ± 0.07^{cA}	6.24 ± 0.20^{bA}	6.76 ± 0.15^{aA}	6.88 ± 0.10^{aB}	
	200 ppm BCWE	3.29 ± 0.03^{eA}	4.50 ± 0.20^{dB}	5.60 ± 0.08^{cA}	6.04 ± 0.07^{bA}	6.72 ± 0.18^{aA}	6.87 ± 0.08^{aB}	
	300 ppm BCWE	3.29 ± 0.04^{fA}	4.41 ± 0.16^{eB}	5.77 ± 0.13^{dA}	6.16 ± 0.36^{cA}	6.72 ± 0.15^{bA}	7.05 ± 0.04^{aA}	
TAPB	Control	3.32 ± 0.03^{fA}	4.71 ± 0.16^{eA}	5.90 ± 0.16^{dA}	6.42 ± 0.16^{cAB}	6.84 ± 0.17^{bA}	7.15 ± 0.11^{aA}	0.089
	100 ppm BCWE	3.30 ± 0.10^{eA}	5.06 ± 0.17^{dA}	6.03 ± 0.14^{cA}	6.34 ± 0.28^{bAB}	6.68 ± 0.10^{aAB}	6.83 ± 0.19^{aB}	
	200 ppm BCWE	3.29 ± 0.08^{eA}	4.88 ± 0.24^{dA}	5.62 ± 0.18^{cB}	6.05 ± 0.25^{bB}	6.61 ± 0.06^{aB}	6.77 ± 0.15^{aB}	
	300 ppm BCWE	3.31 ± 0.07^{eA}	4.72 ± 0.13^{dA}	5.94 ± 0.12^{cA}	6.54 ± 0.19^{bA}	6.60 ± 0.08^{bB}	6.92 ± 0.12^{aAB}	
M/S	Control	3.71 ± 0.14^{abA}	3.89 ± 0.06^{abA}	3.81 ± 0.04^{abA}	3.82 ± 0.12^{abA}	3.96 ± 0.23^{aA}	3.68 ± 0.10^{bA}	0.089
	100 ppm BCWE	3.17 ± 0.28^{cB}	3.54 ± 0.06^{bB}	3.43 ± 0.09^{bC}	3.71 ± 0.11^{abA}	3.93 ± 0.16^{aA}	3.55 ± 0.06^{bAB}	
	200 ppm BCWE	3.45 ± 0.22^{aAB}	3.56 ± 0.11^{aB}	3.59 ± 0.11^{aB}	3.33 ± 0.03^{aB}	3.63 ± 0.29^{aB}	3.40 ± 0.10^{aB}	
	300 ppm BCWE	3.69 ± 0.06^{abA}	3.56 ± 0.04^{bB}	3.59 ± 0.03^{bB}	3.70 ± 0.05^{abA}	3.87 ± 0.14^{aA}	3.63 ± 0.20^{bAB}	
LAB	Control	2.69 ± 0.19^{fA}	3.60 ± 0.08^{eA}	4.14 ± 0.15^{dA}	5.13 ± 0.18^{cA}	5.95 ± 0.22^{bA}	6.55 ± 0.04^{aA}	0.103
	100 ppm BCWE	2.54 ± 0.24^{fA}	3.42 ± 0.15^{eAB}	3.93 ± 0.27^{dAB}	4.82 ± 0.22^{cAB}	5.90 ± 0.16^{bA}	6.53 ± 0.06^{aA}	
	200 ppm BCWE	2.78 ± 0.15^{fA}	3.34 ± 0.16^{eB}	3.62 ± 0.13^{dB}	4.47 ± 0.09^{cB}	5.64 ± 0.09^{bA}	6.47 ± 0.16^{aA}	
	300 ppm BCWE	2.82 ± 0.09^{eA}	3.20 ± 0.09^{eB}	4.01 ± 0.26^{dAB}	4.70 ± 0.37^{cAB}	5.94 ± 0.12^{bA}	6.54 ± 0.23^{aA}	
Pseudomonas	Control	3.40 ± 0.04^{eA}	4.43 ± 0.14^{dB}	4.98 ± 0.11^{cB}	5.73 ± 0.11^{bA}	6.36 ± 0.06^{aA}	6.38 ± 0.07^{aA}	0.067
	100 ppm BCWE	3.37 ± 0.03^{eA}	4.77 ± 0.15^{dA}	5.37 ± 0.13^{cA}	5.80 ± 0.10^{bA}	5.31 ± 0.06^{cD}	6.36 ± 0.22^{aA}	
	200 ppm BCWE	3.38 ± 0.04^{dA}	4.13 ± 0.04^{cC}	4.76 ± 0.09^{bC}	4.98 ± 0.20^{bB}	6.00 ± 0.04^{aC}	6.24 ± 0.24^{aA}	
	300 ppm BCWE	3.37 ± 0.05^{eA}	3.85 ± 0.05^{dD}	5.46 ± 0.02^{cA}	5.77 ± 0.20^{bA}	6.17 ± 0.10^{bB}	6.31 ± 0.07^{aA}	
Enterobacteriaceae	Control	2.27 ± 0.22^{fA}	2.73 ± 0.12^{eA}	3.08 ± 0.04^{dA}	3.54 ± 0.05^{cA}	4.77 ± 0.16^{bA}	5.81 ± 0.03^{aA}	0.111
	100 ppm BCWE	2.11 ± 0.16^{dA}	2.19 ± 0.18^{dB}	2.52 ± 0.07^{cdB}	2.68 ± 0.09^{cB}	3.73 ± 0.11^{bB}	5.21 ± 0.47^{aB}	
	200 ppm BCWE	2.24 ± 0.08^{bA}	2.32 ± 0.22^{bB}	2.36 ± 0.06^{bC}	2.41 ± 0.14^{bC}	3.76 ± 0.27^{aB}	3.90 ± 0.13^{aC}	
	300 ppm BCWE	2.15 ± 0.14^{cA}	2.17 ± 0.14^{cB}	2.51 ± 0.010^{cB}	3.63 ± 0.06^{bA}	4.16 ± 0.51^{aB}	4.20 ± 0.06^{aC}	

Note: Different lowercase letters (a–f) on the same row represent significant differences between storage days for the same treatment ($p < 0.05$).
Different capital letters (A–D) in the same column represent significant differences between treatments on the same storage day ($p < 0.05$).
Abbreviations: LAB, lactic acid bacteria; *M/S*, *Micrococcus/Staphylococcus*; NS, not significant; TAMB, total aerobic mesophilic bacteria; TAPB, total aerobic psychrotrophic bacteria; SEM, standard error of means.

by Degirmencioglu et al.[49] There was no difference ($P > 0.05$) between the treatment groups in terms of TAMB counts during storage except Days 3 and 15. TAMB counts gradually increased in all treatment groups during storage, but the lowest TAMB counts on Day 15 were in the 100 and 200 ppm BCWE groups ($P < 0.05$). The highest TAMB counts during storage were generally determined in the control without BCWE. A similar gradual increasing trend dependent on storage time was also observed for TAPB counts. TAPB counts between 3.29–3.32 log CFU/g on Day 1 reached 6.77–7.15 log CFU/g on the last day of storage. There were no significant differences in TAPB counts between all treatments for the first 3 days of storage ($p > 0.05$), while the lowest counts on Day 6 were found in the 200 ppm BCWE group ($p < 0.05$). On Day 15, the samples containing 100 and 200 ppm BCWE had lower TAPB counts than the control group. These results are consistent with Turan and Turan and Şimşek,[14] who reported that treatment with black mulberry extract reduced total mesophilic and total psychrotrophic bacteria counts in both aerobic and vacuum-packed raw beef patties compared to the control group without extract. Similarly, antimicrobial effects of natural extracts such as black rice, beetroot, pistachio green hull, and nettle on total mesophilic bacteria counts have been reported in meat products.[8,13,22,32]

Regarding the fluctuating *Micrococcus/Staphylococcus* (Gram-positive, catalase-positive cocci) counts during the storage period, lower counts were detected in the BCWE-added treatments during storage compared with the control. The lowest *Micrococcus/Staphylococcus* counts from Day 9 were in the group with 200-ppm BCWE ($p < 0.05$), but similar results were observed in the BCWE groups at the end of storage ($p > 0.05$).

LAB are dominant bacteria in the vacuum-packed fresh meat during storage. It can reach maximum counts in suitable pH values such as 5.5–5.8. LAB counts increased continuously in all groups during storage ($p < 0.05$). The lowest LAB counts at Day 3 compared with the control were 200 and 300 ppm BCWE-added samples, whereas no significant differences were detected between the control and BCWE groups for LAB counts on Days 12 and 15 ($p > 0.05$). These results indicate that the antimicrobial effect of BCWE on LAB growth of vacuum-packed ground beef decreased after Day 9.

Another group of bacteria that grows in ground meat and fresh meat products during cold storage is *Pseudomonas*. In the present study, *Pseudomonas* counts at the beginning of storage (Day 1) ranged from 3.37 to 3.40 log CFU/g, but no significant differences were observed between treatments ($p > 0.05$). Also, *Pseudomonas* counts of ground beef samples with or without BCWE increased significantly ($P < 0.05$) with increasing storage time. The lowest *Pseudomonas* counts on Days 6 and 9 were observed in the 200-ppm BCWE group ($p < 0.05$), but all treatments had similar counts at the end of storage ($p > 0.05$). In a study conducted by Alp and Aksu,[5] it was determined that 250 ppm *Urtica dioica* L. water extract was more effective on total aerobic mesophilic bacteria, *Pseudomonas* and LAB counts of ground beef than 500-ppm concentration.

In the current study, the highest *Enterobacteriaceae* counts during storage were determined in the control group, while the lowest values were found in the BCWE-added groups ($p < 0.05$, Table 4). As the storage time increased, an increase in *Enterobacteriaceae* counts was observed ($p < 0.05$), this increase was greater in the control group without extract. The lowest counts were generally determined in the 200-ppm BCWE group during storage. However, there were no differences between the 200- and 300-ppm BCWE-added groups on Day 15 ($p > 0.05$). *Enterobacteriaceae* counts at the end of storage were 1.91 and 1.61 log CFU/g lower in the 200- and 300-ppm BCWE groups compared with the control. These results showed that BCWE have antibacterial properties on these microorganism. Regarding the shelf life of ground beef, BCWE has the ability to significantly reduce *Enterobacteriaceae* counts. The reducing effect of BCWE on microbial counts is probably due to bioactive compounds, mainly anthocyanins. The antimicrobial activity of anthocyanins can be attributed to mechanisms such as leakage of intracellular material due to disruption of the structural integrity of the wall and membrane, unstable cytoplasmic membrane and poor permeability of the plasma membrane, inhibition of enzymes by interaction with sulphhydryl groups or proteins through oxidized compounds, and deprivation of substrates necessary for microbial growth.[50,51] In addition, anthocyanins can penetrate the inner membrane and inhibit the growth of bacteria by reducing the activity of alkaline phosphatase, adenosine triphosphatase, and superoxide dismutase.[51] Similarly, some studies have reported that black carrot has antimicrobial activity.[4,52] In agreement with the present study, incorporation with black mulberry extracts in vacuum-packed beef patties has been reported to reduce *Enterobacteriaceae* and *Pseudomonas* counts compared with the control without extract.[14] Degirmencioglu et al.[49] found that the counts of *Pseudomonas* and *Enterobacteriaceae* in vacuum-packed ground beef samples stored at 4°C for 7 days ranged from 3.05 to 3.97 log CFU/g and 3.71 to 5.86 log CFU/g, respectively.

4 | CONCLUSION

The present study showed that BCWE has high total phenolic and anthocyanin content as well as significant metal chelating and DPPH radical scavenging activities. The incorporation of BCWE in vacuum-packed ground beef delayed discolouration by significantly inhibiting lipid oxidation and metmyoglobin formation compared to control. Considering *Enterobacteriaceae* counts, the best results for microbial quality were obtained with 200-ppm BCWE concentration. These results indicate that BCWE can be contribute to the shelf life and consumer acceptance of the vacuum-packed ground beef by providing the desired redness, and delaying microbial and oxidative deterioration. In conclusion, BCWE is a very suitable source for use as a food colourant and antioxidant additive and can be recommended as a promising natural extract to reduce the negative effects of vacuum packaging on meat and meat products.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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