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Effects of rosemary and grape seed extracts, ascorbic acid and their combinations on oxidative stability and residual nitrite level in thermally processed ground beef during storage

Auswirkungen von Rosmarin- und Traubenkernextrakten, Ascorbinsäure und deren Kombinationen auf die oxidative Stabilität und den Restnitritgehalt in thermisch verarbeitetem Hackfleisch während der Lagerung

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Summary

This study aimed to investigate the effects of antioxidants (rosemary extract, grape seed extract, ascorbic acid and their combinations) on the residual nitrite level in thermally processed ground beef during storage (0, 1, 7, 15, 30 d) at 4 °C. Cooking loss, pH, color, thiobarbituric acid reactive substances (TBARS), residual nitrite level and texture analysis were performed. Results indicated that incorporation of antioxidants had no effect on cooking loss. Higher TBARS values were determined in control (without any antioxidant) compared to other treatments during storage ($p<0.05$). Rosemary and grape seed extracts were as much effective as nitrite for retarding lipid oxidation in thermally processed ground beef during storage. The residual nitrite level decreased in all treatments during storage ($p<0.05$). The lowest residual nitrite levels were determined in the samples prepared with the combination of ascorbic acid with rosemary extract or grape seed extract during storage ($p<0.05$). It can be concluded that rosemary extract or grape seed extract or their combination with ascorbic acid may be effective strategy to reduce lipid oxidation and residual nitrite level in thermally processed meat products for the meat industry.

Keywords: Rosemary, grape seed, ascorbic acid, extract, residual nitrite, meat

Introduction

Nitrite and nitrate are commonly used preservatives in meat product processing due to antimicrobial activity, especially for inhibition of *Clostridium botulinum*. Also, nitrite has some other technological roles, such as formation and preservation of characteristic curing color in meat products, prevention of lipid oxidation and unique texture and aroma formation (Kim and Hur, 2018). Antimicrobial effect of nitrite is closely related to inhibition of bacterial metabolic enzymes, reduction of oxygen uptake, and disruption of proton exchange (Alahakoon et al., 2015). Also, nitric oxide binds to iron and disrupts the metabolism of microorganisms and the functions of enzymes necessary for their development. Antioxidant capability of nitrite is due to the formation of nitriso- and nitrosyl compounds having antioxidant properties and chelating of free radicals by nitric oxide (Doolaege et al., 2012).

Even though nitrites and nitrates are very important additives in meat products because of those functions metioned above, the risk of formation of carcinogenic substances have created controvery among the meat industry, scientists and consumers (Kilic et al., 2001). To prevent potential health risks posed by nitrites and nitrates in the meat products, strategies have been focused on developing methods in respect to either reducing the initial amount of added nitrite and nitrate in meat product formulations or reducing the amount of residual nitrite or nitrate in the final product (Demeyer et al., 2008). It has been reported that the lower the amount of residual nitrite detected in meat products, the lower the nitrosamine formation and the associated risks to consumer health (Jin et al., 2018). In this perspective, a decreasing the amount of residual nitrite in meat and meat products has been one of the main targets for the meat industry.

Although synthetic antioxidants are still used today, consumer demand for natural antioxidants has increased in recent years due to the possible harmful effects of synthetic antioxidants. For this reason, the recent studies have been targeting to find natural antioxidants (Kılıç et al., 2018). Plants in particular are potential sources of valuable bioactive substances and are considered natural antioxidants to improve the quality of meat and meat products (Shah et al., 2014). To delay oxidative reactions in meat products, some herbal extracts such as green tea, rosemary, grape seed, thyme, pomegranate have started to be used (Aminzare et al., 2019).

Grape (*Vitis vinifera* L.) is one of the most produced fruits in the World (Blanch et al., 2023). It was found that grape and grape derived products contain high levels of phenolic components (Jiang and Xiong, 2016). Since grape seed contains such a high amount of phenolic substances, there has been a main focus on its antioxidant capability. It was reported that grape seed extract has 20 and 50 times more antioxidant capability than vitamin E and vitamin C, respectively (Li et al., 2014). Furthermore, the richest natural polyphenols in its structure were identified as flavonols, phenolic compounds, catechins, proanthocyanidins, and anthocyanins (Karre et al., 2013). Rosemary (*Rosmarinus officinalis* L.) is another important medicinal and aromatic plant (Djenane et al., 2002). It has been stated that rosemary plant has antioxidant, antimicrobial, immune system enhancing and antiviral effects (Nieto et al., 2011). Rosemary extract is used in the meat industry either direct addition into the meat product formulation or adding it into the packaging material (Grumezescu and Holban, 2018). Ascorbic acid is also a widely used reducing and antioxidant additive. Ascorbic acid is able to inactivate pro-oxidant substances and bind reactive oxygen species. However, it is catalyzed by metal ions such as Cu^{+2} and Fe^{+3} and shows an prooxidant effect (Lee et al., 1999). Ascorbic acid used in meat product processing reacts faster with nitrite in a slightly acidic environment compared to secondary and tertiary amines and thus prevents the formation of nitrosamine (Jiang and Xiong, 2016).

The present study aimed to determine the effects of rosemary and grape seed extracts, ascorbic acid and their combinations on the oxidative stability and the residual nitrite level on in thermally processed ground beef during refrigerated storage.

Materials and Methods

Sources of meat and non-meat ingredients

The beef used in this study *(Longissimus thoracis et lumborum)* was sourced from a local slaughterhouse 24 h after slaughter and transported to the laboratory on ice. The beef was procured on three separate occasions. After removing the connective tissue and fat as much as possible, meat was vacuum bagged and kept at –18 °C until used in the production. L(+)-ascorbic acid (Acros Organics, USA), rosemary and grape seed extracts (Immunat Bitkisel İlaç ve Doğal Sağlık Ürünleri A.Ş., Turkey) were obtained commercially.

Preparation of samples

The meat was ground (9.5 mm) by the grinder (Model PKM 22/32, Arı Makine, Istanbul, Turkey), mixed in a bowl mixer (K1292, Arçelik, Istanbul, Turkey) and then reground (3.2 mm). A 10 % water and 1% NaCI addition (the meat weight basis) were applied. An equal amounts of meat samples were divided into treatments, and then antioxidant agents and sodium nitrite were added into each treatment (Table 1). A 45 g meat samples were filled into 50 mL plastic centrifuge tubes. Then, the samples were placed into the water bath with the temperature of 60 ºC and the heat process was initiated by elevating the temperature to 85 ºC. To determine the final internal endpoint temperature, a thermocouple was located in the geometric center of a tube containing 45g ground meat. The heat process continued until reaching the target internal temperature (74 ºC). After cooling the samples to room temperature, cooking loss was determined. The rest of the samples were stored at +4 ºC for 30 d. pH, color, thiobarbituric acid

TABLE 1: *Coding for experimental treatments applied in thermally processed ground beef.*

Treatments	
C	Control (No sodium nitrit,and antioxidant addition)
SN150	150 ppm Sodium nitrite
AA	500 ppm Ascorbic acid
RE	3% Rosemary extract
GSE	3% Grape seed extract
AA150	500 ppm Ascorbic acid + 150 ppm Sodium nitrite
RE150	3% Rosemary extract + 150 ppm Sodium nitrite
GSE150	3% Grape seed extract + 150 ppm Sodium nitrite
AARE	500 ppm Ascorbic acid + 3% Rosemary extract
AAGSE	500 ppm Ascorbic acid + 3% Grape seed extract
AARE150	500 ppm Ascorbic acid + 3% Rosemary extract + 150 ppm Sodium nitrite
AAGSE150	500 ppm Ascorbic acid + 3% Grape seed extract + 150 ppm Sodium nitrite

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reactive substances (TBARS) and residual nitrite (RN) level analysis were performed on processing day and different storage intervals (1, 7, 15 and 30 d).

Cooking loss

The weights of the meat samples were determined before and after heat processing. The cooking loss of the samples was calculated according to the formula shown below (Kılıç et al., 2016).

Cooking loss (%) = 100 x (Raw sample weight – Cooked sample weight) / Raw sample weight

pH

The pH was determined using a spear electrode (FC 200, Hanna Instruments, Germany) attached to a portable pH meter (HI 9024, Hanna Instruments, Germany). Meter was calibrated against 4 and 7 pH buffer standards.

Color

The CIE L *, a *, b * color values were measured on the surface of cooked samples with Precise Color Reader (TCR 200, PCE Instruments, UK). Before measurements, the device was calibrated using its own white calibration plate (Özer and Kılıç, 2015).

Texture

Textural measurements of the meat samples were carried out at room temperature using a TA XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK). The analysis results were evaluated by determining the hardness (N), adhesiveness (mJ), resilience, cohesiveness, springiness, gumminess (N), and chewiness (N) parameters (Bozkurt and Bayram, 2006). Test conditions were; aluminum rectangular probe (5 cm x 4 cm), test speed of 5 mm/s, pre-test speed of 2 mm/s, post-test speed of 2 mm/s, compression of 70%, and 50 kg load cell.

TBARS

TBARS analysis was carried out in the meat samples as described by Kılıç et al. (2018). Two g of sample was weighed and homogenized into 12 mL of the trichloroacetic acid (TCA) extraction solution for 15 s. The homogenized sample was filtered from Whatman 1 filter paper. 1 mL of the obtained filtrate was taken, mixed with 1 mL of thiobarbituric acid (TBA) solution and then vortexed. In the meantime, 1 mL TCA and 1 mL TBA solutions were also prepared as a blank. The mixture was kept at 100 °C for 40 min. After the tubes were cooled in tap water for 5 min and they were centrifuged at 2000 x g for 5 min. The supernatant of the sample was taken and placed in spectro cuvettes and readings were made in the spectrophotometer at 532 nm wavelength. The TBARS values were expressed as µmol TBARS per kg meat.

Residual nitrite

Meat samples (Approximately 5 g) were weighed into beaker and a 40 mL distilled water (80 °C) was added and mixed with the sample. A 350 mL distilled water was added to the mixed sample and the mixture was boiled in the water bath for 2 h and mixed occasionally. The samples were allowed to cool down to room temperature. Then, distilled water was added to the samples to bring the volume to 500 mL. A 5 mL mercuric chloride was added to the liquid in which the sample is present and mixed. After cooling to room temperature, the liquid was filtered. After addition of a 2 mL Griess reagent into the filtrate and the mixture was mixed. Then, it was held in the dark for 1 h for color development and the absorbance values were determined in the spectrophotometer at 520 nm. The residual nitrite levels were calculated by placing the absorbance values into the formula obtained from the standard curve and multiplying the obtained values by the dilution factor 20 (AOAC, 2000).

Statistical analysis

Whole study was carried out as 3 replications and analysis conducted in each replication were also performed in triplicate. The experimental design was completely randomized design with twelve treatments which included eleven treatment groups with sodium nitrite or ascorbic acid or rosemary extract or grape seed extract or various combinations of those and control (No sodium nitrite and antioxidant addition). The results were analyzed by variance analysis (One-way ANOVA) technique using SPSS 22.0.0 (SPSS Inc., Chicago, USA) package program. Data collected for cooking lose, pH, color, textural properties, TBARS and residual nitrite were analyzed by one way analysis of variance to determine significant difference. Significant differences between the average means were tested using the Duncan multiple range test. Differences among mean values were considered significant when $p<0.05$.

Results and discussion

pH and Cooking Loss

The pH values of thermally processed ground beef during storage is shown in Table 2. pH values ranged from 5.83 to 5.97 and from 5.96 to 6.09 on processing day and day 30 respectively. There was an increasing trend ($p<0.05$) in initial pH values of all treatments at the beginning of storage and then pH determined in all treatments were quite stable during the rest of the storage. Similarly, Mokhtar and Youssef (2014) reported an increased pH in beef burgers and authors spaculated that an increased pH might be results of the accumulation of metabolites due to the bacterial action and deamination of proteins. Authors stated that bacteria break down amino acids as the stored glucose is exhausted, ammonia accumulates as the end product of the breakdown of amino acids and pH rises (Mokhtar and Youssef, 2014).

The pH values of AA150, GSE, GSE150, AAGSE, AAGSE150 and AARE treatements on processing day were found to be higher ($p<0.05$) compared to that of control and there was no significant difference among the other treatments. It may be stated that addition of AA and GSE generally caused a pH decrease in the samples compared to control. Patriani and Wahyuni (2022) also reported that AA has acidic nature and lowers the pH of the meat when AA is added into the meat. Zhou et al.(2020) explained that the decreased pH in western-style smoked sausage incorporated with GSE could be due to the presence of organic acids and other acidic compounds in GSE. Hovewer, this decrease was not exist in case of RE. It was stated that the extract of grape seed and skins reduced pH values of chicken meatballs (Nardoia et al., 2017). On the other hand, other studies reported that the addition of grape seed extract did not affect meat pH (Libera et al., 2018). At the end of storage period, AAGSE treatment had lower ($p<0.05$) pH values than the rest of the treatments axcept AA, AARE and AARE150 treatments.

Cooking loss among treatments ranged from 32.32 to 33.92 % (Data is not presented). Cooking loss results indica-

TABLE 2: *pH changes of the thermally processed ground beef treated with natural extracts during storage at 4 °C.*

Treatments	Storage (days)				
	Processing day	1	7	15	30
C	5.97±0.07^{aB}	6.03±0.04^{aA}	6.02±0.01^{cdA}	5.99±0.02^{fAB}	6.04±0.02^{abcdA}
SN150	5.95±0.03^{abC}	6.06±0.01^{aB}	6.10±0.02^{aA}	6.04±0.02^{bcB}	6.06±0.03^{abcB}
AA	5.91±0.07^{abcD}	6.06±0.07^{aAB}	6.07±0.05^{abA}	5.99±0.02^{fC}	6.00±0.02^{defBC}
RE	5.91±0.03^{abcC}	6.07±0.06^{aA}	6.03±0.07^{bcdAB}	6.02±0.01d^{eAB}	5.98±0.03^{defB}
GSE	5.89±0.05^{bcdB}	6.06±0.05^{aA}	6.04±0.04^{bcA}	6.02±0.01^{cdeA}	6.04±0.04^{bcdeA}
AA150	5.88±0.05^{bcdC}	6.05±0.06^{aAB}	6.04±0.04^{bcdB}	6.03±0.01^{bcdB}	6.09±0.01^{aA}
RE150	5.91±0.04^{abcC}	6.05±0.02^{aAB}	6.03±0.04^{bcdB}	6.10±0.02^{aA}	6.08±0.08^{abAB}
GSE150	5.88±0.05^{bcdB}	6.04±0.05^{aA}	6.02±0.01^{cdA}	6.04±0.02^{bA}	6.03±0.04^{bcdeA}
AARE	5.86±0.05^{cdC}	6.06±0.07^{aA}	6.00±0.02^{cdeB}	6.00±0.01^{efB}	6.01±0.01^{cdefAB}
AAGSE	5.83±0.05^{dC}	6.06±0.01^{aA}	5.97±0.01^{eB}	5.99±0.01^{fB}	5.96±0.03^{fB}
AARE150	5.90±0.07^{abcC}	6.06±0.04^{aA}	5.99±0.02^{deB}	6.02±0.01^{cdeAB}	5.98±0.06^{efB}
AAGSE150	5.88±0.05^{bcdB}	6.03±0.02^{aA}	6.02±0.04^{cdA}	6.02±0.02^{cdeA}	6.02±0.07^{bcdeA}

Treatment abbreviations; C: No sodium nitrit,and antioxidant addition, SN: Sodium nitrite, 150: 150 ppm Sodium nitrite, AA: Ascorbic acid, RE: Rosemary extract, GSE: Grape seed extract. $^{a-f}$ Different letters within a column are significantly different (P<0.05). $^{A-D}$ Different letters within a row are significantly different (P<0.05).

of all treatment groups gradually decreased during storage period (p<0.05). It has been previously reported that grape seed extract increased a* values in meat products (Brannan, 2009; Libera et al., 2018). On the other hand, Nardoia et al. (2017) stated that grape seed extract did not change the color values of chicken meatballs.

Results indicated that b * values varied between 3.04 and 5.64 on manufacturing day. While the highest b * values were obtained in RE group, the lowest b * values were determined in AA, AA150, SN150, GSE150 and AAGSE150 groups (p<0.05). At the end of storage, the highest b * values were determined in control, whereas, the lowest b * values were found in AA150, SN150, GSE150 and AAGSE150 groups (p<0.05).

TBARS results

TBARS values of cooked ground beef during storage are presented in Table 4. TBARS values of control increased from 3.18±0.44 µmol/kg during storage and reached the level of 38.68 ± 1.2 µmol/kg at the end of storage. TBARS values of control

ted that the use of natural antioxidants (rosemary extract, grape seed extract, ascorbic acid, and their combinations) and sodium nitrite did not have any effect on cooking loss values among treatments (p<0.05).

Color

Results (Table 3) indicated that the highest L* values were found in AA treatment whereas the lowest values were determined in N150 treatment on processing day (p<0.05). L* values of groups containing only ascorbic acid (AA) or grape seed extract (GSE) were found to be higher than that of control (p<0.05) while other groups had similar values with control. L* values in control increased during the storage period (p<0.05), hovewer, the other experimental groups were quite stable. Effects of grape seed extract on L* values in meat and meat products are quite contraversial. Use of grape seed extract was reported to reduce L* values by Özvural and Vural (2012). On the other hand, Brannan (2009) found that grape seed extract caused an increase L* value whereas some other studies indicated no change in L* values (Aquilani et al., 2018; Libera et al., 2018). In our study, rosemary extract did not affect both L* and a* values. There are also a scientific controversy in the literature regarding effects of rosemary extract on meat color features. Mokhtar and Yousef (2014) reported that rosemary extract increased the L* values, whereas, others indicated that the use of rosemary extract provided color stabilization in various meat products and caused no significant change in color values (Naveena et al., 2013).

As far as a* values are concerned, it was determined that the a* values of the samples formulated with nitrite were higher than others produced without nitrite addition on both manufacturing day and the last day of storage period (p<0.05). On the other hand, even though the lowest a* values were observed in RE, GSE and AAGSE groups on the manufacturing day, the lowest a* values were found in AA, RE, GSE, AARE, AAGSE and control groups at the end of storage (p<0.05). Results revealed that a* values

TABLE 3: *Color changes of the thermally processed ground beef treated with natural extracts at 4 °C.*

Treatments	Storage (days)				
	0	1	7	15	30
L*					
C	56.99±3.79^{cdC}	60.69±1.45^{aB}	60.10±1.63^{abBC}	58.67±1.47^{abBC}	64.42±0.78^{aA}
SN150	54.71±1.15^{dA}	54.76±2.06^{deA}	53.49±1.31^{eA}	53.84±2.03^{eA}	54.61±1.01^{fA}
AA	60.67±1.22^{aC}	58.48±0.93^{bB}	61.14±0.73^{aA}	59.15±1.74^{aBC}	58.85±1.74^{bcC}
RE	56.03±1.73^{cdA}	56.47±2.55^{cdA}	57.32±2.17^{cA}	57.02±1.82^{bcdA}	57.28±0.73^{cdA}
GSE	59.34±1.39^{abA}	58.14±2.16^{bcA}	59.46±1.19^{abA}	58.11±1.84^{abcA}	58.08±1.22^{bcdA}
AA150	54.95±0.66^{dA}	55.17±1.07^{deA}	55.80±0.55^{cdA}	55.67±0.87^{dA}	55.58±1.38^{efA}
RE150	55.20±0.39^{dA}	55.34±0.67^{deA}	55.54±0.73^{cdA}	56.26±1.05^{cdA}	55.47±1.41^{efA}
GSE150	55.10±0.30^{dAB}	54.24±1.37^{eB}	55.66±0.82^{cdA}	55.70±0.51^{dA}	55.13±0.61^{efAB}
AARE	57.08±1.46^{cdA}	56.51±1.95^{cdA}	57.27±2.14^{cA}	56.34±1.99^{cdA}	56.54±1.85^{deA}
AAGSE	57.90±3.18^{bcA}	58.11±1.34^{bcA}	59.08±1.54^{bA}	58.82±1.52^{abA}	59.37±1.60^{bA}
AARE150	54.85±0.92^{dB}	55.12±0.61^{deAB}	55.24±1.72^{dAB}	56.42±0.49^{cdA}	56.52±1.33^{deA}
AAGSE150	54.75±1.52^{dA}	55.54±0.53^{deA}	55.44±1.37^{dA}	55.73±0.89^{dA}	55.19±1.68^{efA}
a*					
C	12.75±1.86^{cdA}	11.95±1.51^{cAB}	10.60±1.97^{dB}	11.50±1.08^{dAB}	8.40±0.26^{fC}
SN150	26.61±0.62^{aA}	25.55±0.89^{aA}	23.75±0.63^{bcB}	23.79±0.70^{bcB}	24.03±1.56^{bcB}
AA	13.35±0.87^{cA}	11.92±0.89^{cB}	10.95±1.55^{dB}	11.69±0.51^{dB}	10.88±1.60^{dB}
RE	10.73±1.64^{efA}	9.48±0.67^{eB}	9.19±0.08^{eB}	9.17±0.26^{fB}	9.07±0.51^{efB}
GSE	11.92±1.74^{deA}	10.40±0.73^{deB}	9.89±0.68^{deB}	9.96±0.41^{eB}	10.48±0.68^{dB}
AA150	26.11±0.49^{aA}	25.44±1.09^{aAB}	25.57±0.60^{aAB}	24.82±0.72^{aBC}	24.22±1.46^{abC}
RE150	24.41±0.62^{bA}	23.87±0.94^{bAB}	23.24±0.83^{cAB}	23.26±0.28^{cdAB}	22.75±1.84^{cB}
GSE150	24.76±0.84^{bB}	25.67±0.98^{aA}	23.47±0.34^{cC}	23.06±0.33^{cC}	23.55±0.78b^{cC}
AARE	10.20±1.40^{fA}	9.34±0.57^{eB}	9.21±0.24^{eB}	8.99±0.29^{fB}	9.07±0.34^{efB}
AAGSE	11.19±1.25^{efA}	10.71±0.95^{dAB}	10.01±0.31^{deB}	10.09±0.16^{eB}	10.29±0.41^{deAB}
AARE150	26.33±0.54^{aA}	25.57±0.73^{aA}	24.71±0.33^{abB}	23.82±0.52^{bcC}	24.01±0.91^{bcBC}
AAGSE150	26.21±0.43^{aA}	26.47±0.65^{aA}	25.28±0.47^{aB}	24.36±0.53^{abC}	25.39±0.62^{aB}
b*					
C	4.87±1.26^{bcB}	4.65±0.58^{bB}	5.40±0.97^{aB}	4.47±0.55^{bB}	7.54±0.94^{aA}
SN150	3.29±0.56^{fgAB}	2.61±0.42^{dC}	2.58±0.37^{eC}	2.68±0.42^{dBC}	3.71±0.5^{efA}
AA	3.44±0.60^{efgB}	4.41±0.70^{bcAB}	3.99±1.58^{bcB}	3.79±0.39^{cB}	5.45±1.22^{cA}
RE	5.64±0.65^{aA}	5.51±0.19^{aB}	5.76±0.53^{aA}	5.42±0.52^{aA}	5.67±0.82^{bcA}
GSE	4.10±0.62^{efBC}	3.95±0.24^{cB}	4.68±0.29^{bA}	4.18±0.32^{bcB}	5.08±0.32^{cdA}
AA150	3.21±0.47^{fgA}	2.79±0.19^{dA}	3.01±0.42^{deA}	2.98±0.32^{dA}	3.20±0.41^{fA}
RE150	4.24±0.27^{bcdeA}	4.24±0.36^{bcA}	4.29±0.12^{bA}	3.98±0.42^{bcA}	4.42±0.39^{dA}
GSE150	3.04±0.43^{gAB}	3.22±0.74^{dAB}	2.99±0.30^{deAB}	2.80±0.71^{dB}	3.59±0.68^{efA}
AARE	4.99±0.47^{bD}	5.22±0.14^{aCD}	6.04±0.20^{aAB}	5.68±0.37^{aBC}	6.38±0.57^{bA}
AAGSE	4.00±1.25^{cdefA}	4.26±0.82^{bcA}	4.26±0.45^{bA}	4.01±0.43^{bcA}	4.60±0.39^{dA}
AARE150	4.37±0.47^{cdAB}	4.35±0.28^{bcAB}	4.64±0.34^{bA}	4.14±0.36^{bcB}	4.62±0.38^{dA}
AAGSE150	3.52±0.50^{defgA}	2.91±0.48^{dB}	3.46±0.35^{cdA}	3.14±0.16^{dAB}	3.64±0.39^{efA}

Treatment abbreviations; C: No sodium nitrit,and antioxidant addition, SN: Sodium nitrite, 150: 150 ppm Sodium nitrite, AA: Ascorbic acid, RE: Rosemary extract, GSE: Grape seed extract. $^{a-g}$ Different letters within a column are significantly different (P<0.05). $^{A-D}$ Different letters within a row are significantly different (P<0.05).

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group were higher than that of all other groups during whole storage period ($p<0.05$). TBARS values of the samples with nitrite addition were found to be lower than control during storage ($p<0.05$). It was also previously reported that the TBARS values determined in sausages with nitrite addition were lower than those without nitrite (Feng et al., 2016; Jin et al., 2018). Study results revealed that using only ascorbic acid (AA group) in formulation was insufficient to prevent lipid oxidation development in cooked ground beef samples after 7 days of storage. Even though TBARS values of control were the highest, AA group had the second highest TBARS values among all treatment groups starting from day 7 to until the last day of storage ($p<0.05$). Similarly, Sánchez-Escalante et al. (2001) reported that ascorbic acid was insufficient to completely neutralize lipid oxidation in a modified atmosphere packaged meatballs. Furthermore, researchers stated that the antioxidant effects of ascorbic acid changes depending on the dose used and its antioxidant capability is affected by the metal ions and tocopherol content of muscle foods. On the other hand, using only rosemary (RE group) or grape seed (GSE group) extracts provided more effective results regarding inhibition of TBARS formation compared to using ascorbic acid alone ($p<0.05$). Hovewer, it was observed that elevated TBARS formation determined in the samples incorporated with only ascorbic acid group (AA) was able to be reduced when ascorbic acid was combined with nitrite or rosemary extract or grape seed extract ($p<0.05$). The reason for this is thought to be related to individual antioxidant capabilities of nitrite, rosemary extract and grape seed extract used. It was previously emphasized that grape seed extract has high antioxidant properties as it contains high amounts of phenolic components in its structure (Al-Hijazeen et al., 2019). Phenolic compounds showed antioxidant properties by transferring the hydrogen atoms in their structures to radicals (Jongberg et al., 2013). It has been stated that the antioxidant activity of rosemary extract was based on the functioning of the phenolic diterpenes in the structure as hydrogen donor or holding free radical components (Al-Hijazeen et al., 2019). It was also reported that the antioxidant mechanism of rosemary extract resembles other polyphenols and flavonoids and the presence of a catechol group in the aromatic ring of the phenolic diterpene skeleton of rosemary is probably the most important structural element in antioxidant activity (Shan et al., 2005).

TABLE 4: *TBARS (mg malonaldehyde/kg) values of the thermally processed ground beef treated with natural extracts during storage.*

Treatments	Storage (days)				
	0	1	7	15	30
C	3.18 ± 0.44^{aD}	11.32 ± 0.93^{aC}	27.64 ± 2.28^{aB}	35.30 ± 1.46^{aA}	38.68 ± 1.2^{aA}
SN150	0.48 ± 0.14^{bA}	0.63 ± 0.02^{cA}	0.62 ± 0.10^{cA}	0.72 ± 0.17^{cA}	1.08 ± 0.48^{cA}
AA	0.65 ± 0.10^{bC}	1.54 ± 0.69^{bcC}	16.92 ± 2.17^{bB}	20.50 ± 4.10^{bB}	27.65 ± 3.22^{bA}
RE	0.56 ± 0.06^{bB}	1.23 ± 0.67^{cA}	0.70 ± 0.09^{cAB}	0.70 ± 0.13^{cAB}	1.10 ± 0.45^{cAB}
GSE	0.50 ± 0.07^{bD}	0.70 ± 0.08^{cCD}	0.89 ± 0.27^{cBC}	1.03 ± 0.25^{cAB}	1.20 ± 0.18^{cA}
AA150	0.54 ± 0.07^{bB}	0.60 ± 0.11^{cB}	0.52 ± 0.06^{cB}	0.64 ± 0.05^{cAB}	0.83 ± 0.27^{cA}
RE150	0.59 ± 0.04^{bB}	0.67 ± 0.05^{cAB}	0.69 ± 0.05^{cAB}	0.62 ± 0.11^{cB}	0.76 ± 0.09^{cA}
GSE150	0.57 ± 0.05^{bBC}	0.93 ± 0.38^{cAB}	1.04 ± 0.43^{cA}	0.48 ± 0.05^{cC}	1.05 ± 0.25^{cA}
AARE	0.60 ± 0.08^{bB}	0.67 ± 0.15^{cB}	0.67 ± 0.05^{cB}	0.72 ± 0.10^{cB}	0.89 ± 0.39^{cA}
AAGSE	0.61 ± 0.06^{bB}	0.63 ± 0.22^{cB}	0.89 ± 0.08^{cB}	0.89 ± 0.09^{cB}	1.84 ± 0.68^{cA}
AARE150	0.63 ± 0.06^{bA}	0.70 ± 0.12^{cA}	0.75 ± 0.19^{cA}	0.60 ± 0.12^{cA}	0.88 ± 0.39^{cA}
AAGSE150	0.55 ± 0.05^{bAB}	0.64 ± 0.10^{cAB}	0.60 ± 0.13^{cAB}	0.48 ± 0.08^{cAB}	0.78 ± 0.33^{cA}

Treatment abbreviations; C: No sodium nitrit,and antioxidant addition, SN: Sodium nitrite, 150: 150 ppm Sodium nitrite, AA: Ascorbic acid, RE: Rosemary extract, GSE: Grape seed extract. $^{a-c}$ Different letters within a column are significantly different ($P<0.05$). $^{A-D}$ Different letters within a row are significantly different ($P<0.05$).

TABLE 5: *Changes in residual nitrite (mg/kg) in the thermally processed ground beef treated with natural extracts during storage.*

Treatments	Storage (days)				
	0	1	7	15	30
C	0.91 ± 0.08^{eBC}	1.18 ± 0.10^{cB}	1.65 ± 0.08^{dA}	0.55 ± 0.29^{fC}	1.69 ± 0.47^{dA}
SN150	14.53 ± 1.77^{bcA}	14.85 ± 1.41^{aA}	14.73 ± 0.14^{aA}	13.13 ± 1.19^{aA}	10.79 ± 1.10^{aB}
AA	0.67 ± 0.22^{eC}	1.02 ± 0.11^{cAB}	1.12 ± 0.08^{dAB}	0.75 ± 0.11^{fBC}	1.25 ± 0.35^{dA}
RE	0.78 ± 0.11^{eB}	1.06 ± 0.07^{cA}	1.27 ± 0.11^{dA}	1.18 ± 0.11^{fA}	1.25 ± 0.28^{dA}
GSE	0.61 ± 0.35^{eB}	0.95 ± 0.08^{cB}	1.06 ± 0.15^{dA}	0.63 ± 0.08^{fB}	1.09 ± 0.04^{dA}
AA150	15.20 ± 1.24^{abA}	14.31 ± 1.92^{aA}	11.20 ± 2.09^{bB}	10.42 ± 1.84^{cBC}	7.79 ± 1.89^{bC}
RE150	16.71 ± 0.77^{aA}	14.64 ± 1.37^{aA}	12.13 ± 2.23^{bB}	12.17 ± 1.24^{abB}	11.08 ± 0.86^{aB}
GSE150	16.57 ± 1.26^{aA}	15.39 ± 0.56^{aA}	12.15 ± 3.26^{bB}	11.54 ± 0.65^{bB}	8.29 ± 0.81^{bC}
AARE	1.36 ± 0.47^{eAB}	0.65 ± 0.07^{cC}	0.86 ± 0.22^{dBC}	0.92 ± 0.14^{fBC}	1.60 ± 0.75^{dA}
AAGSE	1.22 ± 0.33^{eA}	0.63 ± 0.08^{cBC}	1.00 ± 0.13^{dAB}	0.43 ± 0.08^{fC}	1.11 ± 0.63^{dAB}
AARE150	13.16 ± 1.99^{cdA}	10.80 ± 1.90^{bB}	8.20 ± 1.21^{cC}	$8.87\pm^{dBC}$	5.97 ± 1.05^{cD}
AAGSE150	12.19 ± 1.85^{dA}	9.74 ± 0.92^{bB}	8.91 ± 0.84^{cBC}	7.67 ± 086^{eC}	4.78 ± 1.01^{cD}

Treatment abbreviations; C: No sodium nitrit,and antioxidant addition, SN: Sodium nitrite, 150: 150 ppm Sodium nitrite, AA: Ascorbic acid, RE: Rosemary extract, GSE: Grape seed extract. $^{a-e}$ Different letters within a column are significantly different ($P<0.05$). $^{A-B}$ Different letters within a row are significantly different ($P<0.05$).

Residual nitrite

Table 5 shows the changes in the amount of residual nitrite levels during 30 d storage. Residual nitrite levels varied from 0.61 to 16.71 mg/kg among treatments at the beginning of the storage. As expected, higher residual nitrite levels were observed in treatment groups with added nitrite (SN150, AA150, RE150, GSE150, AARE150 and AAGSE150) compared to those without nitrite addition at the beginning of the storage ($p<0.05$). The amount of residual nitrite in the groups without nitrite addition was determined between 0.61–1.36 mg/kg and did not differ among these groups.

The residual nitrite levels in these nitrite added treatment groups decreased during 30 days of storage ($p<0.05$). At the end of storage, the amount of residual nitrite in SN150, RE150, AA150, GSE150, AARE150 and AAGSE150 groups decreased by 25.7, 33.7, 48.8, 50, 54.6 and 60.8%, respectively, compared to the production day. In this respect, some previous studies also reported that the amount of residual nitrite in cured muscle foods decreased during storage because of oxidation (Liu et al., 2010; Li et al., 2012). It was also stated a decrease in the residual nitrite level during storage was associated with type of meat, meat pH, the amount of initial nitrite, the production and storage temperature, and the reducing substances exist in the environment (Xi et al., 2012).

At the end of storage, the highest residual nitrite levels were determined in SN150 and RE150 groups followed by AA150 and GSE150 groups which had higher residual than AARE150 and AAGSE150 groups ($p<0.05$). It was observed that addition of either ascorbic acid or grape seed extract into meat which was formulated with 150 mg/kg sodium nitrite were able to reduce residual nitrite level more effectively compared to those produced with only 150 mg/kg sodium nitrite ($p<0.05$). No significant pH differences were determined

between control and group in which ascorbic acid was added. It has been previously reported that ascorbic acid was capable of reducing the amount of residual nitrite without pH change (Choi et al., 2017). Similarly, some previous studies have reported that ascorbic acid was able to reduce residual nitrite levels in muscle foods (Choi et al., 2017; Kim and Hur, 2018). Our study results also revealed that using a combination of ascorbic acid and grape seed extract in meat containing 150 mg/kg sodium nitrite was even more effective for reducing residual nitrite in final product ($p<0.05$). Karwowska and Kononiuk (2020) suggested that direct chemical interaction between antioxidants and nitrites/nitrates has long been recognized and they suggested that antioxidants have reduced nitrite toxicity. In a similar study, it was assumed that antioxidants and residual nitrite are closely related and thus antioxidants delaying lipid oxidation may similarly reduce the residual nitrite level (Hah et al., 2006). The lowest residual nitrite levels in the present study were obtained in control and other groups produced without sodium nitrite addition ($p<0.05$). Li et al. (2012) investigated the effect of green tea and grape seed polyphenols and ascorbic acid on residual nitrite levels in dried cured sausages and authors indicated that plant polyphenols and ascorbic acid significantly reduced the amount of residual nitrite, while ascorbic acid was the most effective additive. Choi et al. (2017) added red beet extract and ascorbic acid combinations into emulsion-type meat product and researchers revealed that red beet extract reduced residual nitrite, but they observed that this effect was even stronger when red beet extract was used with ascorbic acid.

As far as rosemary extrac is concerned, present study revealed that using the rosemary extract alone in the formulation created 33.7% decrease in the initial residual nitrite level at the end of storage, whereas this rate increased to 54.6% when it was used with ascorbic acid. It has been stated that the reduction of residual nitrite in cured meat may have resulted from a pH decline caused by rosemary extract addition, since decreasing the product pH increases the formation of nitric oxide from nitrite (Jin et al., 2018). Ascorbic acid addition to a cured meat product was reported to create acceleration in the formation of nitric oxide from nitrite which can result in declined residual nitrite levels in the product (Vossen et al., 2012).

Texture analysis results

The effects of antioxidant addition on textural properties of cooked ground beef are presented in Table 6. Results indicated that all experimental groups showed similar hardness, cohesiveness and springiness characteristics with each other. Even though adhesiveness, resilience, gumminess and chewiness features of the experimental groups were quite similar, these textural parameters showed some variations among some experimental groups. In this regard, RE150 group had higher adhesiveness and lower resilience values compared to AARE group ($p<0.05$). Control had the highest ($p<0.05$) gumminess values. Higher chewiness values were determined in AA group than AA150 group ($p<0.05$). Özvural and Vural (2012) examined the effect of grape seed extract on sausages and reported that hardness, resilience, chewiness values remained unchanged, while adhesiveness values increased, gumminess values decreased. Gadekar et al. (2014) reported in their study that the natural antioxidants used in restructured goat meat did not change the textural features other than cohesiveness.

Conclusions

In our study, the use of ascorbic acid, natural extracts (rosemary and grape seed), and their combinations was evaluated as an alternative method to reduce residual nitrite in processed meat products. Results revealed that these natural extracts had as much as high antioxidant activity sodium nitrite in cooked ground beef. The result indicated that residual nitrite decreased with the incorporation of ascorbic acid or grape seed extract in cooked ground beef. Although rosemary extract alone was not sufficient for reducing residual nitrite effectively, the combination of ascorbic acid and rosemary extract significantly reduced the amount of residual nitrite. The amount of residual nitrite was found to be the lowest when rosemary and grape seed extracts were combined with ascorbic acid. As a result of the study, it is recommended for the meat industry to use rosmery or grape seed extracts or their combinations with ascorbic acid to have effective residual nitrine reduction and prolonged shelf life in ready-to-eat meat products.

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Conflict of Interest

Authors do not have any conflict of interests to disclose nor do they endorse the use of any product/technology/service over the other.

TABLE 6: *Textural changes of thermally processed ground beef treated with natural extracts.*

Treatments	Hardness (N)	Adhesiveness (mJ)	Resilience	Cohesiveness	Springiness	Gumminess (N)	Chewiness (N)
C	5.91±1.07^{ab}	0.48±0.30^{abc}	0.12±0.02^{ab}	0.51±0.04^{a}	0.87±0.04^{a}	4.01±0.51^{a}	2.63±0.56^{ab}
SN150	6.84±1.52^{a}	0.50±0.08^{abc}	0.13±0.01^{ab}	0.52±0.02^{a}	0.87±0.06^{a}	3.02±0.10^{b}	2.77±0.77^{ab}
AA	6.75±2.32^{a}	0.53±0.24^{abc}	0.13±0.03^{ab}	0.50±0.04^{a}	1.11±0.40^{a}	2.97±1.13^{b}	3.09±1.18^{a}
RE	5.81±0.87^{ab}	0.35±0.17^{bc}	0.12±0.01^{ab}	0.50±0.02^{a}	1.10±0.48^{a}	2.68±0.28^{bc}	2.74±0.17^{ab}
GSE	5.65±1.28^{ab}	0.35±0.13^{bc}	0.12±0.02^{ab}	0.52±0.06^{a}	0.83±0.06^{a}	2.71±0.27^{bc}	2.40±0.57^{ab}
AA150	4.52±0.75^{a}	0.38±0.25^{abc}	0.13±0.04^{ab}	0.51±0.09^{a}	0.87±0.04^{a}	2.26±0.17^{bc}	1.99±0.21^{b}
RE150	5.84±1.10^{ab}	0.70±0.32^{a}	0.09±0.01^{b}	0.50±0.02^{a}	0.86±0.03^{a}	2.49±0.05^{bc}	2.49±0.60^{ab}
GSE150	5.82±0.95^{ab}	0.28±0.10^{bc}	0.12±0.01^{ab}	0.48±0.03^{a}	0.89±0.04^{a}	2.36±0.25^{bc}	2.48±0.43^{ab}
AARE	6.26±1.12^{a}	0.23±0.21^{c}	0.14±0.03^{a}	0.53±0.07^{a}	0.84±0.01^{a}	2.90±0.72^{b}	2.78±0.72^{ab}
AAGSE	5.57±1.15^{ab}	0.30±0.08^{bc}	0.12±0.03^{ab}	0.52±0.04^{a}	0.95±0.17^{a}	3.00±0.15^{b}	2.69±0.45^{ab}
AARE150	5.06±1.46^{ab}	0.60±0.12^{ab}	0.10±0.02^{ab}	0.48±0.05^{a}	0.91±0.04^{a}	2.00±0.25^{c}	2.20±0.72^{ab}
AAGSE150	6.22±1.06^{ab}	0.38±0.21^{abc}	0.12±0.03^{ab}	0.48±0.04^{a}	0.86±0.11^{a}	2.70±0.34^{bc}	2.53±0.46^{ab}

Treatment abbreviations; C: No sodium nitrit,and antioxidant addition, SN: Sodium nitrite, 150: 150 ppm Sodium nitrite, AA: Ascorbic acid, RE: Rosemary extract, GSE: Grape seed extract. $^{a-c}$ Different letters within a column are significantly different ($P<0.05$).

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