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Effects of thymol and rosemary essential oils and red beet extract on low-nitrite and carmine-free beef Mortadella

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Abstract

In the present study, essential oils were used in order to reduce the nitrite in beef Mortadella, and the red beet extract was used instead of carmine. Beef Mortadella samples containing thymol and rosemary essential oils and red beet extract were produced. Chemical, physical, sensorial, and microbiological analyses were performed during the storage period. The antimicrobial and antioxidant effects of nitrite can be achieved by using thymol and rosemary essential oils. Thiobarbituric acid values of nitrite-free samples were found to be higher than nitrite-containing samples. Redness values of samples containing red beet extract and samples containing nitrite were highest. It can be recommended to use rosemary essential oil and red beet extract in reduced-nitrite and carmine-free meat products. The rosemary essential oil was reported to be more acceptable than thymol from sensorial aspect, although thymol has high antimicrobial and antioxidant effects and high phenolic content when compared with rosemary essential oil.

Novelty impact statement:

- To investigate the effects of nitrite-reduced and carmine-free beef Mortadella samples at the same time with two different essential oils and red beet extract and with different quality characteristics,
- To rank the general tastes with multiple decision-making techniques of sensory analysis,
- To compare the quality characteristics of the samples.

1 | INTRODUCTION

The main functions of nitrite, which is the most frequently used additive in meat products, are to develop typical taste and aroma of cured meat, to prevent lipid oxidation, to create the typical color of cured meat production by reacting with myoglobin, and to produce an antimicrobial effect (Marco et al., 2006). However, since nitrite causes the formation of carcinogenic N-nitroso compounds, the studies concentrated on other additives (Zarringhalami et al., 2009).

Studies were carried out on the alternative additives, which fulfill various functions of nitrate and nitrite in the processed meat products such as antimicrobial, anti-oxidative, and colorization effects

formation and which pose a lower level of health risk in comparison to nitrate and nitrite (Öztürk et al., 2015). Since nitrate/nitrite has various functions in meat, no single additive that could fulfill all these functions could be found. Besides decreasing or preventing the use of nitrite in meat products by making use of natural elements, organic acids, irradiation, pressure applications, and hazard technology were also investigated in the previous studies (Turp & Sucu, 2016).

Thyme is an important herb, thanks to its high thymol concentration. Previous studies revealed the anti-oxidative, antimicrobial, expectorant, antispasmodic, and antibacterial effects of thymol (Amiri, 2012; Höferl et al., 2009; Sharifi-Rad et al., 2017). Among the volatile oils used in the food industry, rosemary (Rosmarinus officinalis L.) oil is widely used in order to expand the shelf-life of foods. The studies reported the beneficial effects of essential oil of rosemary as a natural antioxidant preventing the color degradation and/or lipid oxidation of food (Balentine et al., 2006; Hussain et al., 2010). With its phenolic compound and betalain contents, red beet is considered as a good antioxidant source and natural colorant (Ravichandran et al., 2013). Betalains, which are among the main compounds that red beet extract contains, are more resistant to pH change in comparison with anthocyanin used as natural pigments to obtain red-purple color in foods (Roy et al., 2004).

Since the color is an important criterion for achieving the visual quality in meat products, carmine is widely used as a colorant material in the emulsified meat products. However, it was reported that the consumers have concerns about carmine-containing meat products, as with nitrite-containing ones (Greenhawt & Baldwin, 2009). In parallel with the consumer expectations, the natural colorant materials that might be used in meat products were investigated in many studies. Researchers emphasized that, because of its strong phenolic content, red beet can be used as a natural colorant in low-nitrite emulsified sausages (Jin et al., 2014).

In the present study, it was aimed to use the red beet extract and essential oils together in low-nitrite beef Mortadella to observe the changes in microbiological, chemical, physical, and sensorial characteristics of products during 90-day shelf-life and to provide an alternative additive profile.

2 | MATERIAL AND METHOD

2.1 | Supplies and chemicals

The meat and animal fat used in the present study were procured from a company based in Sivas province, whereas thymol (Thymol-T0501) and rosemary (Rosemary Oil-W299200) essential oil were purchased from Sigma and red beet extract from TGS Chemistry (İstanbul). Thymol and rosemary essential oils were 99% pure, certified, and fat-soluble.

The beef Mortadella samples used in the present study were prepared in a local meat production facility in Sivas. The formulations of commercially available were accepted as a model for the experimental samples. Beef Mortadella paste was prepared using beef rib meat (65%-Yıldız Meat, Sivas), animal fat (10%-Yıldız Meat, Sivas), ice (15%), spice mixture (2%-Selay, İstanbul), starch (5%-Selay, İstanbul), soy protein (2.5%-Selay, İstanbul), salt (1.7%-Sivas Salt, Sivas), and fume aroma (0.1%-Selay, İstanbul).

2.2 | Preparation of beef Mortadella samples and experimental design

Meat-fat mixture was processed using a bowl cutter (Vemag, Denmark) at low speed (100 rpm) for 20–30 s, and then ice, spice mixture (red pepper powder, cumin powder, black pepper powder),

maltodextrin, dextrose monohydrate, and polyphosphate were added. The mixture was then processed at gradually increasing speeds (2,000, 3,000, and 3,500 rpm) in bowl cutter (Vemag– Denmark). Finally, the process in the cutter was continued at maximum speed (5,500 rpm) until achieving a good emulsion consistency according to emulsion formation of the study in Verma et al. (2016). The experiment groups were established using preliminary trials. According to the sensorial and instrumental color analyses, 1% red beet extract was added into the samples in order to compare with the commercial control group (0.02% carmine). About 0.3% (w/w) concentration was preferred for both essential oils. Twelve experimental groups were established using red beet extract, thymol, and rosemary essential oils determined in preliminary trials (Table 1).

The emulsion mixtures were transferred from cutter to vacuum filling machine (Handtmann VF 610–Germany) and filled into 38Q-diameter plastic casings (Fibran–Spain) with 200 g constant portioning. The samples were cooked in an automatic steam oven for 40 min at 100% moisture and 20 min at 60% moisture. When the internal temperature reached 72°C, they were rapidly cooled after resting for 10 min. Five beef Mortadella samples were produced for each treatment for every storage period and replication. The beef Mortadella samples were stored at 4°C for 0, 7, 14, 30, 60, and 90-day storage periods. Experiments were performed in triplicate with different occasions and different meat samples. Analyses were triplicated for each replicate $(n = 3 \times 3)$.

2.3 | Analyses

2.3.1 | Chemical analyses

pH levels of beef Mortadella samples were measured using Hanna HI-2221 pH-meter, which was calibrated with its three standard buffers (4.01-7.01-10.01) and had temperature control system (at 20–22°C; Vural & Öztan, 1996).

The number of thiobarbituric acid (TBA) was measured with the spectrophotometric method by modifying the methods of Tarladgis et al. (1960) and Jin et al. (2014).

Free radical scavenging effect of samples on DPPH was determined by revising the method recommended by Brandwilliams et al. (1995).

Total phenolic content was determined using the Folin-Ciocalteu method applied by Zadernowski et al. (2009). Total phenolic content was measured using the gallic acid calibration curve prepared with different concentrations (Zadernowski et al., 2009).

2.3.2 | Instrumental color analyses

The instrumental color measurements of samples were performed with Minolta Spectrophotometer CM-3600d by using Hunter-Lab

TABLE 1 Experimental sample formulations

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| | | Type and concentrat | | |
|-------------|--------------------------|---------------------|------------------------|----------------|
| Sample code | Nitrite content (ppm) | Thymol % (w/w) | Rosemary EO % (w/w) | Colorant (%) |
| C0 | 0 | 0 | 0 | 0 |
| C1 | 150 | 0 | 0 | Carmine (0.02) |
| Т | 0 | 0.3 | 0 | O ^a |
| R | | 0 | 0.3 | 0 ^a |
| E | | 0 | 0 | 1 ^a |
| TE | | 0.3 | 0 | 1 ^a |
| RE | | 0 | 0.3 | 1 ^a |
| NT | 75 | 0.3 | 0 | 0 ^a |
| NR | | 0 | 0.3 | 0 ^a |
| NE | | 0 | 0 | 1 ^a |
| NTE | | 0.3 | 0 | 1 ^a |
| NRE | | 0 | 0.3 | 1 ^a |

Notes: C0: Contains no nitrite, essential oil, and colorant. C1: Contains 150 ppm nitrite and 0.02% carmine. T: Contains 0.3% (w/w) thymol. R: Contains 0.3% (w/w) rosemary essential oil. E: Contains 1% red beet extract. TE: Contains 0.3% (w/w) thymol and 1% red beet extract. RE: Contains 0.3% (w/w) rosemary essential oil and 1% red beet extract. NT: Contains 75 ppm nitrite and 0.3% (w/w) thymol. NR: Contains 75 ppm nitrite and 0.3% (w/w) rosemary essential oil. NE: Contains 75 ppm nitrite and 1% red beet extract. NTE: Contains 75 ppm nitrite and 1% red beet extract. NTE: Contains 75 ppm nitrite and 1% red beet extract. NTE: Contains 75 ppm nitrite, 0.3% (w/w) rosemary essential oil, and 1% red beet extract. NRE: Contains 75 ppm nitrite, 0.3% (w/w) rosemary essential oil, and 1% red beet extract. a^aRed beet extract.

color scale and L^* , a^* , and b^* values were determined (Candogan & Kolsarici, 2003) by its CM-A106 target mask and 10° standard observer. The parameters of illuminants were primary D65–Daylight 6500 Kelvin, secondary A–Tungsten, tertiary F2–cool white fluorescent with 2,400 bps baud rate from three random different surfaces of the samples. The device was calibrated prior to the measurement by using a standardized white tile.

2.3.3 | Texture profile analyses

The texture of beef Mortadella samples was analyzed using Texture Analyzer (Ametek Lloyd Instruments, England) device and Warner Bratzler cutting knife set. The test speed was set to 200 mm/min, trigger value to 0.05 N, compression rate to 50%, and sample crosssection length to 15 mm. The changes in hardness 1, hardness 2, cohesiveness, gumminess, flexibility, and chewiness were observed (Cierach et al., 2009).

2.3.4 | Microbiological analyses

Numbers of total psychrophilic aerobic bacteria, lactic acid bacteria (LAB), yeast/mold, and total coliform in beef Mortadella samples were analyzed according to the method of Halkman (2005).

2.3.5 | Sensory analyses

The samples were rated by 18 trained panelists selected from the personnel and graduate students of Food Engineering Department at Sivas Cumhuriyet University. Beef Mortadella samples were cut into small pieces (20 \pm 0.5 mm thickness) and immediately served to the panelists. Panelists used a 5-point hedonic scale testing the color, appearance, odor, and taste (5 = perfect, 1 = very bad). Sensory analyses were carried out with two repetitions. Simple additive weighting (SAW) was used in calculating the general appreciation scores of samples with different weights for different criteria. SAW technique is a method used in finding the most liked sample in cases where more than one criterion is found according to Figure 1 (Afshari et al., 2010). This method gives a comparison between each criterion. The first step is construction of pairwise comparison matrix $(m \times n)$ based on Saaty's 1-9 scale (equally preferred, equally to moderately,, very strongly to extremely, extremely preferred). Selection of the important criteria for each comparison is the second step. Construction of a decision matrix $(m \times n)$ including m alternative and n criteria. Normalization of the decision matrix as step 3 in AHP method. Building of the weighted normalized matrix with the following equation:

$$A_i = \sum w_i x_{ij}$$



FIGURE 1 Hierarchy decision process of beef Mortedalla samples

where x_{ij} is the score of the *i*th alternative with respect to the *j*th criteria and w_j is the weight of the criteria. Calculating the sum of the weighted normalized vectors to find out the ranking of the alternatives (Dogan et al., 2016).

2.3.6 | Statistical analyses

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pH, TBA, DPPH, total phenolic content, color parameters (*L**, *a**, *b**), texture profile parameters (hardness 1, hardness 2, cohesiveness, gumminess, flexibility, chewiness), and microbiological results data were analyzed separately in one-way ANOVA by using IBM SPSS 23.0 package software. The mean values of triplicated measurements were taken. Fixed factors for all models included nitrite concentration (0–75–150 ppm), red beet concentration (0%–1%), and essential oils (thymol 0%–0.3% and rosemary essential oil 0%–0.3%). The statistically significant differences between groups were subjected to Tukey's HSD (honestly significant difference) test (p < .05). Moreover, the differences in sample groups between the days (0, 7, 14, 30, 60, and 90th day) were analyzed using general linear model-repeated measures analysis. The differences obtained were determined using the Bonferroni test (Adler & Parmryd, 2010).

3 | RESULTS AND DISCUSSION

3.1 | pH values

pH values of beef Mortadella samples ranged between 6.00 and 6.24 on 0th day and between 5.79 and 6.24 on the 90th day. The

pH values of beef Mortadella samples did not change significantly during the storage period (p > .05; Table 2). Similarly, in a study, pH values were obtained during the shelf-life of Mortadella including different concentration thymol and rosemary essential oils, and pH values did not change statistically (Viuda-Martos, 2011). In the study, pH values of C1, E, TE, NR, NE, and NTE were decreased compared to the 0th and 90th days. This agrees with the observations of Dykes et al. (1991) who suggested that the decrease in pH during the storage of meat products is due to the action of LAB. For the emulsified pork sausage samples prepared by decreasing nitrite concentration and using different concentrations (0.5% and 1%) of red beet extract, Jin et al. (2014) reported that red beet changed the pH value. The same authors also emphasized that pH values of all the samples added with red beet extract were higher than that in the control group and that pH values increased together with the increasing amount of red beet extract addition. Turp et al. (2016) determined that there was no significant difference between the pH values of sausages prepared by adding red beet powder but, when compared with the control sample, pH values of sausages added with red beet powder were higher than the control sample.

3.2 | TBA values

The thiobarbituric acid (TBA) values of beef Mortadella samples are presented in Figure 2. The number of TBA is the expression of fat oxidation as malonaldehyde (MA), and it was reported that the maximum acceptable value in meat products should be 1 mgMA/kg sample (Ockerman, 1985). Among the beef Mortadella samples, only the 90th-day TBA number of CO group (containing no nitrite, essential oil, or red beet extract; 1.079 mgMA/kg sample) exceeded that limit. TBA numbers of samples ranged between 0.255 and 0.339 mgMA/ kg sample on the 0th day. The highest TBA number during the storage period was found in CO sample, whereas the lowest values were found in NTE and NRE sample. TBA number of C1 was found to be higher than that of these samples (NTE and NRE). With the effect of nitrite, the nitrite-free samples' (T, R, E, TE, and RE) TBA numbers on the 90th day of storage were found to be higher than those of nitrite-containing samples (NT, NR, NE, NTE, and NRE). In a previous study, the researchers added red beet extract, ascorbic acid, and Na-lactate to pork sausage and reported that this combination decreased TBA level and prolonged the shelf-life of product (Martínez et al., 2006). In another study, 0%, 2%, 4%, and 6% red beet extracts were added to sausage samples, and TBA numbers were examined during the storage period. It was reported that the samples containing red beet extract had lower TBA numbers in comparison with the control samples containing no extract (Turp et al., 2016).

3.3 | DPPH free radical scavenging activity results

During the storage period, the highest DPPH values were found to be in NRE sample on 0th day, NRE sample on 7th day, C1 sample on Journal of Food Processing and Preservation

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 TABLE 2
 pH and DPPH radical scavenging activity (% inhibition) values changes in beef Mortedalla samples stored at 4°C

| | | Storage period (da | ay) | | | | |
|--------------|-------------------|----------------------------|----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|
| Sample | | 0 | 7 | 14 | 30 | 60 | 90 |
| pН | C0 | $6.14\pm0.09^{\text{ab}}$ | 6.29 ± 0.09^{a} | 6.27 ± 0.01^{d} | $6.28\pm0.01^{\text{a}}$ | $6.16\pm0.12^{\text{a}}$ | $6.14\pm0.09^{\text{a}}$ |
| | C1 | $6.15\pm0.09^{\text{ab}}$ | $6.24\pm0.07^{\rm a}$ | 6.27 ± 0.00^{d} | $6.20\pm0.09^{\text{a}}$ | $6.24\pm0.07^{\text{a}}$ | 5.95 ± 0.26^{a} |
| | Т | 6.17 ± 0.04^{ab} | $6.22\pm0.08^{\text{a}}$ | 6.23 ± 0.03^{bcd} | $6.24\pm0.01^{\text{a}}$ | $6.29\pm0.14^{\text{a}}$ | $6.17\pm0.07^{\text{a}}$ |
| | R | $6.11\pm0.01^{\text{ab}}$ | $6.17\pm0.07^{\text{a}}$ | $6.22\pm0.01^{\text{abcd}}$ | $6.27\pm0.06^{\text{a}}$ | $6.20\pm0.04^{\text{a}}$ | $6.17\pm0.06^{\text{a}}$ |
| | Е | 6.24 ± 0.15^{b} | $6.19\pm0.11^{\text{a}}$ | $6.24 \pm 0.02 \text{ cd}$ | $6.27\pm0.05^{\text{a}}$ | $6.14\pm0.16^{\text{a}}$ | $5.98\pm0.02^{\text{a}}$ |
| | TE | $6.00\pm0.04^{\text{a}}$ | $6.14\pm0.09^{\text{a}}$ | $6.15\pm0.03^{\text{a}}$ | $6.20\pm0.06^{\text{a}}$ | $6.08\pm0.00^{\text{a}}$ | $5.83\pm0.23^{\text{a}}$ |
| | RE | 6.01 ± 0.09^{ab} | $6.15\pm0.06^{\text{a}}$ | 6.16 ± 0.04^{ab} | $6.20\pm0.05^{\text{a}}$ | $6.19\pm0.02^{\text{a}}$ | $6.17\pm0.09^{\text{a}}$ |
| | NT | 6.11 ± 0.06^{ab} | $6.26\pm0.02^{\text{a}}$ | $6.19\pm0.04^{\text{abc}}$ | $6.29\pm0.02^{\text{a}}$ | $6.21\pm0.06^{\text{a}}$ | $6.11\pm0.08^{\text{a}}$ |
| | NR | 6.11 ± 0.07^{ab} | $6.23\pm0.06^{\text{a}}$ | $6.25 \pm 0.02 \text{ cd}$ | $6.33\pm0.05^{\text{a}}$ | $6.11\pm0.09^{\text{a}}$ | $5.87\pm0.07^{\text{a}}$ |
| | NE | $6.00\pm0.05^{\text{a}}$ | $6.15\pm0.07^{\text{a}}$ | $6.17\pm0.01^{\rm abc}$ | 6.24 ± 0.00^{a} | $6.14\pm0.09^{\text{a}}$ | 5.79 ± 0.39^{a} |
| | NTE | 6.14 ± 0.00^{ab} | $6.20\pm0.11^{\text{a}}$ | $6.22\pm0.01^{\text{abcd}}$ | $6.22\pm0.01^{\text{a}}$ | $6.22\pm0.00^{\text{a}}$ | $6.08\pm0.01^{\text{a}}$ |
| | NRE | 6.05 ± 0.09^{ab} | $6.17\pm0.04^{\text{a}}$ | $6.19\pm0.01^{\text{abc}}$ | $6.24\pm0.02^{\text{a}}$ | $6.24\pm0.03^{\text{a}}$ | $6.24\pm0.03^{\text{a}}$ |
| DPPH radical | PPH radical CO 51 | $51.57 \pm 20.60^{\times}$ | $52.83 \pm 20.51^{\times}$ | $49.56 \pm 15.68^{\times}$ | 52.61 ± 18.43^{x} | $45.08\pm21.01^{\times}$ | 41.49 ± 24.36^{x} |
| scavenging | C1 | 73.99 ± 4.44^{xy} | 72.99 ± 4.54^{xy} | 77.14 ± 5.59^{9} | 78.15 ± 7.11^{xy} | 76.90 ± 7.22^{9} | $78.10\pm6.19^{\rm y}$ |
| activity | Т | 67.53 ± 6.01^{xy} | 72.560 ± 4.32^{xy} | 76.63 ± 4.67^{9} | 70.96 ± 7.30^{xy} | 73.78 ± 6.50^{9} | $73.24\pm8.63^{\text{y}}$ |
| | R | 70.15 ± 4.31^{xy} | 68.69 ± 4.28^{xy} | 71.47 ± 4.77^{9} | 77.57 ± 4.63^{xy} | 74.79 ± 4.82^{9} | 72.32 ± 7.43^{9} |
| | Е | 69.99 ± 5.05^{xy} | 75.02 ± 4.72^{xy} | $72.29 \pm 4.81^{\text{y}}$ | 71.55 ± 4.28^{xy} | $74.86\pm5.11^{\text{y}}$ | $75.40 \pm 7.13^{ m y}$ |
| | TE | 56.48 ± 19.77^{xy} | 72.32 ± 5.39^{xy} | 71.90 ± 4.77^{9} | 68.01 ± 15.08^{xy} | 73.69 ± 7.88^{9} | 71.08 ± 8.97^{xy} |
| | RE | 74.60 ± 5.01^{xy} | 74.41 ± 5.05^{xy} | 69.25 ± 4.23^{xy} | 76.22 ± 8.29^{xy} | 75.77 ± 6.76^{9} | 68.73 ± 4.27^{xy} |
| | NT | 62.36 ± 4.02^{xy} | 69.20 ± 6.33^{xy} | 68.12 ± 6.67^{xy} | 66.48 ± 4.05^{xy} | 69.70 ± 5.04^{xy} | 66.58 ± 4.52^{xy} |
| | NR | 74.37 ± 5.03^{xy} | 73.15 ± 4.731^{xy} | 74.09 ± 4.72^{9} | 74.77 ± 7.07 ^{×y} | 78.95 ± 5.79^{9} | $73.36\pm5.85^{\text{y}}$ |
| | NE | 68.31 ± 5.25^{xy} | 74.73 ± 4.70^{xy} | $70.64 \pm 4.73^{ m y}$ | 78.60 ± 4.94^{xy} | $73.79 \pm 9.21^{\text{y}}$ | $76.69 \pm 5.71^{\text{y}}$ |
| | NTE | 72.77 ± 7.73^{xy} | 74.31 ± 4.87^{xy} | $74.54\pm5.44^{\text{y}}$ | $80.44\pm5.07^{\text{y}}$ | $76.35 \pm 9.15^{\text{y}}$ | 72.67 ± 4.79^{9} |
| | NRE | $75.60 \pm 7.45^{ m y}$ | 76.42 ± 8.64^{9} | 76.20 ± 7.88^{9} | 76.95 ± 8.44^{xy} | 79.39 ± 6.86^{9} | 73.55 ± 9.16^{9} |

Note: $a^{-d, x-y}$ The difference between the samples represented with the different letters in the same column is significant (p < .05).



FIGURE 2 TBA changes of beef Mortedalla samples stored at 4°C. Measurements were taken at 0, 7, 14, 30, 60, and 90th days. Significant differences between means are denoted by superscripts. Letter differences indicate that superscripts were generated on an individual TBA (mg malonaldehyde/kg sample) basis using model-based Tukey's HSD

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14th day, NTE sample on 30th day, NRE sample on 60th day, and C1 sample on 90th day. On all the analysis days, the lowest DPPH values were observed in C0 group (Table 2). DPPH values of C0 group ranged between 41.49% and 52.83% during the storage period. It can be stated that the reason why DPPH values in other sample groups were higher than in C0 group might be the nitrite, thymol, rosemary oil, or red beet extract contents in other groups.

In a study examining the DPPH values of sausages containing thyme, rosemary, and oregano extracts during 90-day storage period, it was reported that the DPPH values of extract-containing samples were higher than that in control group, and the highest DPPH value was found in the sample containing all three extracts (Bayrak, 2011). In another study examining the DPPH values of sausage samples containing 0.1% and 0.2% thyme and rosemary during storage at 10°C, it was reported that DPPH of samples added with thyme and rosemary on 0th day has increased (Jin et al., 2016). DPPH values of both sausage samples containing 0.1% and 0.2% thyme were found to be 90%, whereas DPPH values of sausage samples containing 0.1% and 0.2% rosemary were found to be 65% and 89%, respectively. At the end of 6-week period, it was observed that DPPH values of thyme-containing samples decreased from 90% to 80%, and DPPH values of samples containing 0.1% and 0.2% rosemary were found to decrease to 58% and 88%, respectively (Jin et al., 2016). When compared with the present study, DPPH results were similar, and the differences might be because the materials used were plants or the storage period was 4°C in the present study but -18°C in the study of Jin et al. (2016).

3.4 | Total phenolic content results

During the storage, the lowest total phenolic content (TPC) was found to range between 81.05 and 278.53 mg gallic acid/ml sample. However, the highest values were found to be between 1,767.39 and 2,313.30 mg gallic acid/ml sample (Figure 3). During the storage period, the lowest TPC was found in the group containing low nitrite but thymol and red beet extract (NTE). When comparing the beef Mortadella samples by the type of essential oil they contain (T-R, TE-RE, NT-NR, and NTE-NRE), it was found that, during the storage period, the TPC of thymol-containing groups (T, TE, NT, and NTE) was found to be higher than that of groups containing rosemary essential oil (R, RE, NR, and NRE). Besides that, when compared with the control group (C0) and the group-containing red beet extract (E), it was determined that the change in TPC during the storage period was statistically nonsignificant (p > .05).

In a previous study, mandarin peel powder extract, pomegranate peel powder extract, and pomegranate seed powder extract were added into goat-meat meatballs and the TPC were analyzed; the TPC of samples were reported to be 900, 1,200, and 590 μ g/g (Devatkal et al., 2010). In their study, Ekici et al. (2015) measured the TPC of experimental soujuck samples containing decreased nitrite and different concentrations (0.5%, 1%, and 2%) of black carrot concentrate. According to the results obtained, they reported that the TPC of soujuck samples containing black carrot extract was higher than that in commercial control sample containing carmine. TPC of samples containing 2% black carrot concentrate were reported to



FIGURE 3 Total phenolic contents of beef Mortedalla samples stored at 4°C. Measurements were taken at 0, 7, 14, 30, 60, and 90th days. Significant differences between means are denoted by superscripts. Letter differences indicate that superscripts were generated on an individual total phenolic contents (mg gallic acid/ml sample) basis using model-based Tukey's HSD

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be 1,055–1,201 mg gallic acid/mL sample, and it was also determined that TPC increased with the increasing amount of black carrot concentrate.

3.5 | Instrumental color (L^* , a^* , b^*) results

In all the storage durations, the lowest brightness values (L^*) of samples were found in C1 as seen in Table 3. During the storage, L^* values C1, T, R, RE, NT, NR, and NTE samples were statistically increased (p < .05). Similarly, in a study carried out by Bayrak (2011), the sausages prepared with mechanically deboned chicken meat, rosemary, and thyme were analyzed in terms of color values during the shelf-life. The author concluded that the L^* values of all the samples were increased during the storage, and the highest brightness value was found in thyme-containing samples. Moreover, it was reported that L^* values of control sausage samples on 0th day increased from 50.69 to 56.35 on the 90th day, that of rosemary-containing samples increased from 50 to 55.49, and that of thyme-containing samples

increased from 51.50 to 57.31. These findings are in parallel with those reported in the literature.

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It was reported that the most important instrumental color parameter of meat products is a^* (redness), and it is related with oxygenation of myoglobin (Sirocchi et al., 2017). High a^* values suggest the presence of oxymyoglobin and low values suggest the formation of metmyoglobin (Esmer et al., 2011). a* values of samples decreased during the storage, but there was no statistically significant difference (p > .05). a^* values of T and R (containing no red beet) groups were found to be lower than a^* values of TE and RE (containing red beet extract) and NT and NR (containing nitrite) groups (Figure 4). It can be said that red beet extract and nitrite positively effected the a^* values. It was reported that, thanks to its betalain content, the red beet extract plays an effective role in the increase in redness values of meat products (Ravichandran et al., 2012). Similar to the present study, Jeong et al. (2010) reported that, during the storage period, a* values of sausage samples containing red beet extract decreased depending on the pigment degradation. However, the authors also emphasized that the redness values of samples containing 75 ppm

TABLE 3 The changes in L^* and b^* values of beef Mortedalla samples stored at 4°C

| | | Storage period (days) | | | | | | |
|-----------|-----|-----------------------------|----------------------------------|--------------------------------|---------------------------------|--------------------------------|--------------------------------|--|
| Sample | | 0 | 7 | 14 | 30 | 60 | 90 | |
| L* values | C0 | 56.89 ± 3.18^{a} | 57.78 ± 2.70^{ab} | 58.64 ± 3.16^{ab} | 59.04 ± 2.80^{ab} | 59.53 <u>+</u> 3.43 | 59.90 ± 3.25^{b} | |
| | C1 | 51.97 ± 1.67^{aA} | 52.03 ± 1.44^{aA} | 52.67 ± 1.57^{aA} | 52.77 ± 1.11^{aA} | 53.31 ± 1.51^{B} | 53.27 ± 1.60^{aB} | |
| | т | 57.62 ± 2.29^{bA} | 58.53 ± 2.32^{bA} | 59.37 ± 2.41^{bB} | 59.38 ± 2.49^{bB} | 59.71 ± 2.74^{B} | 59.98 ± 3.03^{bB} | |
| | R | 55.81 ± 0.62^{aA} | 56.67 ± 0.74^{abA} | 56.66 ± 0.56^{abA} | 57.32 ± 0.69^{abB} | 57.65 ± 0.16^{B} | 58.26 ± 0.93^{abB} | |
| | Е | 54.58 ± 5.47^{a} | 56.32 ± 4.66^{ab} | 56.44 ± 4.36^{ab} | 57.11 ± 4.05^{ab} | 57.44 ± 4.08 | 59.14 ± 2.60^{ab} | |
| | TE | 53.86 ± 1.16^{a} | 54.83 ± 1.25^{ab} | 55.75 ± 1.31^{ab} | 55.87 ± 1.54^{ab} | 56.16 ± 2.51 | 57.11 ± 1.71^{ab} | |
| | RE | 53.67 ± 1.26^{aA} | $54.64 \pm 1.06^{\text{abA}}$ | 54.91 ± 1.70^{abA} | 55.58 ± 2.03^{abA} | 56.37 ± 3.07^{A} | 57.47 ± 2.12^{abB} | |
| | NT | 57.41 ± 1.56^{aA} | 57.94 ± 1.57^{abA} | 58.07 ± 2.08^{abA} | $58.83 \pm 1.71^{\text{abA}}$ | $58.33 \pm 2.51^{\text{A}}$ | 59.34 ± 1.64^{abB} | |
| | NR | 56.96 ± 2.49^{aA} | 57.24 ± 2.12^{abA} | 57.94 ± 2.34^{abA} | 58.56 ± 2.92^{abA} | $58.41 \pm 3.86^{\text{A}}$ | 59.37 ± 3.00^{abB} | |
| | NE | $53.08 \pm 1.07^{\text{a}}$ | 54.08 ± 1.34^{ab} | 53.47 ± 1.47^{ab} | 54.75 ± 1.99^{ab} | 56.03 ± 1.96 | 56.37 ± 1.58^{ab} | |
| | NTE | 54.58 ± 0.92^{aA} | 54.35 ± 1.41^{abA} | 55.05 ± 1.58^{abA} | $55.50 \pm 1.44^{\text{abB}}$ | 56.44 ± 0.77^{B} | 56.23 ± 0.96^{abB} | |
| | NRE | $54.10\pm2.08^{\text{a}}$ | 54.75 ± 1.36^{ab} | 54.82 ± 0.83^{ab} | 55.94 ± 1.27^{ab} | 55.62 ± 2.36 | 56.98 ± 0.94^{ab} | |
| b* values | C0 | 16.93 ± 1.39^{z} | 18.00 ± 0.91^z | 17.57 ± 0.98 ^y | $17.71 \pm 0.13^{\circ}$ | 17.65 ± 0.21^{x} | $17.59 \pm 0.46^{\circ}$ | |
| | C1 | 8.24 ± 0.25^t | 8.74 ± 0.21^t | 8.95 ± 0.23^t | 9.33 ± 0.68^t | 9.69 ± 0.83^{t} | 9.82 ± 0.91^{t} | |
| | Т | 16.52 ± 0.79^{yz} | 16.69 ± 0.70^{yz} | 17.08 ± 0.58^{vy} | 16.69 ± 0.22^{vy} | 16.74 ± 0.25^{ux} | 16.73 ± 0.22^{xv} | |
| | R | 15.03 ± 1.57^{xvyzT} | $15.81 \pm 1.58^{\text{vyTU}}$ | 16.25 ± 1.89^{xvyTU} | 16.17 ± 1.39^{xvyTU} | $16.49 \pm 1.70^{\text{uxTU}}$ | $16.50 \pm 1.61^{\text{uxvU}}$ | |
| | Е | 15.47 ± 2.11^{vyz} | 16.48 ± 1.02^{yz} | 15.42 ± 1.89^{uxvy} | 16.40 ± 0.34^{xvy} | 15.97 ± 0.73^{ux} | $17.45 \pm 0.56^{\circ}$ | |
| | TE | 14.21 ± 0.42^{uxvyz} | $15.67 \pm 1.04^{\text{xvy}}$ | 15.69 ± 0.59^{uxvy} | 15.26 ± 0.19^{uxv} | $16.11\pm0.68^{\text{ux}}$ | 15.97 ± 0.56^{uxv} | |
| | RE | 13.65 ± 0.60^{uxvT} | $14.67 \pm 0.39^{\text{uxvyTU}}$ | 15.33 ± 0.33^{uxvyTU} | $15.83 \pm 0.55^{\text{uxvTU}}$ | 15.46 ± 0.40^{uTU} | 15.78 ± 0.54^{uxvU} | |
| | NT | 13.40 ± 0.32^{uxv} | 14.25 ± 0.32^{uxv} | 14.38 ± 0.61^{uxv} | 14.75 ± 0.47^{ux} | $15.16\pm0.65^{\rm u}$ | 15.49 ± 0.50^{ux} | |
| | NR | 13.79 ± 0.59^{uxvyT} | $14.53 \pm 0.35^{\text{uxvyTU}}$ | $14.34 \pm 0.71^{\text{uxTU}}$ | $15.58 \pm 0.51^{\text{uxvU}}$ | $15.49 \pm 1.01^{\text{uxTU}}$ | 16.01 ± 0.40^{uxvU} | |
| | NE | 12.04 ± 0.32^{u} | $13.02\pm0.22^{\text{u}}$ | 13.58 ± 0.40^{ux} | $14.30\pm0.05^{\rm u}$ | $14.79\pm0.12^{\rm u}$ | $14.77\pm0.10^{\rm u}$ | |
| | NTE | 12.91 ± 0.29^{uxv} | $13.15\pm0.13^{\rm u}$ | $13.96\pm0.18^{\text{ux}}$ | 14.79 ± 0.54^{ux} | $14.68\pm0.35^{\text{u}}$ | $14.97\pm0.18^{\text{ux}}$ | |
| | NRE | 12.27 ± 0.32^{ux} | $13.64\pm0.28^{\text{ux}}$ | 13.51 ± 0.30^{u} | 14.44 ± 0.41^{u} | $15.04\pm0.40^{\text{u}}$ | $15.24\pm0.22^{\text{ux}}$ | |

Note: ^{a,b,t-2}The difference between the samples represented with the different letters in the same column is significant (p < .05). ^{A,B-T,U}The difference between the samples represented with the different letters in the same row is significant (p < .05).





FIGURE 4 Changes redness (*a**) of beef Mortedalla samples stored at 4°C. Measurements were taken at 0, 7, 14, 30, 60, and 90th days. Significant differences between means are denoted by superscripts. Letter differences indicate that superscripts were generated on an individual redness (*a**) basis using model-based Tukey's HSD

sodium nitrite and 0.5% red beet remained stable. In a study carried out with experimental pork sausages prepared by decreasing the nitrite concentration and adding red beet extract at different concentrations, it was determined that a^* values of sausage samples containing 1% red beet extract were 10 times higher than those of control group containing no red beet extract (Jin et al., 2014).

 b^* values of beef Mortadella samples changed during the storage period, but the changes in b^* values of groups (except for those in R, RE, and NR group) were found to be statistically nonsignificant (p > .05). On all the analysis days, the lowest b^* values were observed in C1 group (8.24-9.82), and the difference between all the groups was found to be statistically significant (p < .05; Table 3). In a previous study, it was determined that b^* value decreased as the amount of red beet powder in experimental sausage samples added with red beet powder at different concentrations increased and b^* value decreased in all the sausages stored at 4°C for 60 days. In the same study, b^* values of sausage samples containing 0, 2, 4, and 6% red beet powder on 0th day were found to be 15.21, 13.35, 11.90, and 10.79, respectively (Turp et al., 2016). In the present study, however, b^* values of samples containing 1% red beet powder extract on 0th day were found to range between 12.04 and 15.47. These results are in parallel with those reported by Turp et al. (2016).

3.6 | Texture profile analysis results

It was determined that the hardness 1 values of beef Mortadella samples tended to increase during the storage period; hardness 1

value was found to range between 21.71 and 31.14 N on 0th day and between 31.35 and 41.54 N on the 90th day (Table 4). Based on this result, it can be stated that hardness 1 values of samples containing thymol did not change during the storage period. Examining the hardness 2 values of beef Mortadella samples, as in hardness 1 values, the hardness 2 values of samples tended to increase (Table 4). Examining the cohesiveness values, the differences between the cohesiveness values of CO-NE-NTE groups and C1-T-E-NR groups on 0th day and the differences between the cohesiveness values of R-RE groups and E-TE-NT-NRE groups on the 90th day (p < .05; Table 4). Examining the flexibility values of beef Mortadella samples, it was found that the flexibility of samples increased during the storage, and it can be seen statistically (p < .05). Examining the gumminess characteristics of beef Mortadella samples, it was found that the gumminess values of CO, T, R, RE, and NR samples increased when compared with 0th and 90th days. Examining the chewiness values of beef Mortadella samples on 0th and 90th days of storage, increases were observed in all the groups, except for NT group (Table 4). The chewiness values of samples were found to range between 80.03 and 149.98 on 0th day and between 131.19 and 192.79 on the 90th day. Examining the chewiness values of beef Mortadella samples, it was found that the differences between chewiness values of groups other than C1, E, NE, and NTE groups were statistically significant (p < .05).

In their study, Estévez et al. (2006) examined the texture values of sausages added with rosemary essential oil at different concentrations (150, 300, and 600 ppm) during the storage period. They reported that hardness groups increased in all the groups, and the

| | Hardness 1 (N | (7 | Hardness 2 (N | (7 | Cohesiven | ess | Flexibility (m | m) | Gumminess | (N) | Chewiness (Nm | (L |
|-----------------------------|------------------------|----------------------|------------------------|---------------------|--------------------|----------------------|-----------------------|-----------------------|-----------------------------|---------------------|------------------------|-----------------------|
| Groups | Oth day | 90th day | Oth day | 90th day | Oth day | 90th day | Oth day | 90th day | Oth day | 90th day | Oth day | 90th day |
| CO | 22.04 ^{abA} | 31.59^{aB} | 18.30 ^{aX} | 26.49 ^{aY} | 0.20 ^a | 0.22 ^{bcd} | 19.01 ^{abK} | 19.36 ^{aK} | 4.34 ^{aS} | 6.71 ^{abT} | 80.03 ^{aF} | 131.19 ^{aG} |
| C1 | 25.58 ^{abcA} | 33.30 ^{aB} | 22.68 ^{abcX} | 28.83 ^{aY} | 0.31^{b} | 0.23 cd | 16.04 ^{aK} | 19.96 ^{abL} | 8.01 ^{cS} | 7.94 ^{bS} | 120.82^{abcF} | 159.74 ^{abG} |
| F | 24.69 ^{abcA} | 31.35^{aA} | 21.96^{abcX} | 27.60 ^{aY} | 0.25 ^b | 0.22 ^{abcd} | 17.22 ^{aK} | 19.69 ^{abL} | 6.18 ^{abcS} | 7.01 ^{abT} | 107.39 ^{abcF} | 135.23 ^{bG} |
| Я | 24.38 ^{abcA} | 31.87 ^{aBC} | 21.35^{abcX} | 28.97 ^{aY} | 0.24 ^{ab} | 0.26 ^d | 16.63 ^{aK} | 19.35 ^{al} | 5.91 ^{abcS} | 8.16 ^{cT} | 94.58 ^{abF} | 157.57 ^{abG} |
| ш | 21.71 ^{aA} | 37.13 ^{aA} | 18.97^{abX} | 28.93 ^{aY} | 0.26 ^b | 0.18 ^{abc} | 18.19 ^{abK} | 22.03 ^{abcL} | 5.89 ^{abcS} | 6.73 ^{abS} | 100.06 ^{abF} | 149.12^{abG} |
| TE | 27.31^{abcdA} | 35.69 ^{aA} | 23.83 ^{abcdX} | 28.50 ^{aX} | 0.22 ^{ab} | 0.16 ^a | 21.51 ^{bK} | 24.05 ^{cK} | 6.00 ^{abcS} | 5.73 ^{aS} | 128.22^{bcF} | 137.46 ^{abF} |
| RE | 30.25 ^{abcdA} | 36.10 ^{aB} | 25.32 ^{bcdX} | 31.94^{aV} | 0.23 ^a | 0.25 ^d | 18.46 ^{abK} | 21.61 ^{abcL} | 6.66 ^{abcS} | 8.74 ^{cT} | 116.92^{abcF} | 186.46 ^{bG} |
| NT | 31.14 ^{cdA} | 35.59 ^{aA} | 27.41 ^{cdX} | 29.55 ^{aY} | 0.24 ^{ab} | 0.17 ^{ab} | 19.71 ^{bK} | 23.03 ^{bcL} | 7.59 ^{bcT} | 5.96 ^{aS} | 148.33 ^{cG} | 136.29 ^{aF} |
| NR | 30.17 ^{abcdA} | 41.54^{aB} | 25.98 ^{cdX} | 32.82 ^{aX} | 0.26 ^b | 0.20 ^{abcd} | 19.66 ^{bKL} | 22.93 ^{abcL} | 7.39 ^{bcS} | 8.71 ^{cT} | 137.76 ^{bcG} | 191.04 ^{bG} |
| NE | 35.05 ^{dA} | 39.79 ^{aB} | 29.74 ^{dX} | 32.30 ^{aX} | 0.20 ^a | 0.18 ^{abc} | 21.59 ^{bK} | 23.23 ^{bcL} | 6.97 ^{abcS} | 7.25 ^{bcS} | 149.98^{cG} | 168.16 ^{abG} |
| NTE | 23.22 ^{abcA} | 38.11 ^{aA} | 18.79 ^{abX} | 30.93 ^{aX} | 0.22 ^a | 0.21 ^{abcd} | 22.22 ^{bK} | 24.16 ^{cl} | 4.90 ^{abS} | 7.99 ^{cS} | 110.94^{abcG} | 192.79 ^{bF} |
| NRE | 30.69 ^{bcdA} | 38.74 ^{aB} | 26.68 ^{cdX} | 31.96 ^{aX} | 0.25 ^b | 0.17 ^a | 18.63 ^{abK} | 23.97 ^{cl} | 7.56 ^{bcT} | 6.48 ^{abS} | 136.84^{bcF} | 153.80 ^{abF} |
| Note: ^{a-f} The di | ference betwee | en the samples re | spresented with | h the different le | tters in the s | ame column is s | significant ($p < 0$ | 05). A.B-X,Y-K,L-S,T | ^{-F,G} The differe | nce between the | e samples represer | ted with the |

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different letters in the same row is significant (p < .05)

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hardness of samples containing rosemary essential oil were lower than that in the control group. It was also determined in the present study that the hardness values of samples increased during the storage period. Various researchers reported that the increase in hardness values of emulsion structures during the storage in a refrigerator is related with the emulsion destabilization process because of the segregation of water and oil from protein matrix. Besides this, the oxidation of protein affects the functionality and emulsification ability of protein (Xiong, 2000). In the same study carried out by Estévez et al. (2006), it was also reported that the cohesiveness and flexibility values of samples containing rosemary essential oil were lower than those of control group samples. Similarly, Jeong et al. (2010) carried out a study with fat-reduced sausages and reported that the addition of red beet did not yield a change in textural properties of sausages. While it was reported in a study that addition of 0-1,000 ppm nitrite into minced canned jambon did not affect the texture (Randall & Voisey, 1977), it was also reported in another study that tomato peel and tomato powder added into pork meatloaf improved the texture properties (Hayes et al., 2013).

3.7 Microbiological analysis results

Among the beef Mortadella samples, the LAB were found to be below the detectable level (<1 log CFU/g) C1 and samples containing essential oil during the storage at 4°C. LAB growth was observed in CO and E (0.25 and 0.07) since 14th day, and the number of LAB increased during the storage in both groups. As the storage time continues, besides CO and E, containing no nitrite and essential oil LAB growth was also observed in NE-containing essential oil and reduced nitrite on the 90th day. The numbers of LAB in CO, E, and NE on the 90th day were found to be 2.41, 1.80, and 1.42 log CFU/g (Table 5).

In a previous study, the crawfish meat marinated with thyme and rosemary essential oils were stored at 4°C for 10 days, and at the end of the storage period, the LAB numbers of samples containing rosemary and thyme essential oils were found to be lower than that of control sample (Duman et al., 2012). In that study, LAB numbers of samples containing rosemary and thyme essential oils were 5.91 and 4.93 log CFU/g, respectively, and it was reported that the thyme essential oil was found to be more effective on the inhibition of LAB when compared with the rosemary essential oil. In the present study, the yeast and mold (YM) levels of samples until the 90th day of beef Mortadella samples' storage were found to be below the detectable level, and YM development was observed on the 90th day in the samples other than C1, NT, NR, and NTE. The difference between the groups, in which YM development was observed, was found to be statistically significant (p < .05). The differences between CO and T, R, E, and NRE were statistically significant (p < .05), whereas the difference between CO and other groups was found to be statistically nonsignificant (p > .05). Among the beef Mortadella samples, the sample having the highest number of YM was C0 (2.58 log CFU/g). In a previous study, the number of YM in control sample at the end of 45-day storage of vacuumed chicken sausages containing different

Changes in hardness 1 (N) and hardness 2 (N) values of beef Mortedalla samples stored at 4°C

TABLE 4

 TABLE 5
 Changes in the number of LAB in beef Mortedalla samples stored at 4°C (log CFU/g)

| | Storage peri | od (days) | | | | | | | |
|--------|--------------|-----------|---------------------------|--------------------|---------------------------|---------------------------|--|--|--|
| Sample | 0 | 7 | 14 | 30 | 60 | 90 | | | |
| C0 | <1 | <1 | 0.25 ± 0.03^{bA} | 1.80 ± 0.24^{bB} | 2.28 ± 0.31^{bC} | 2.41 ± 0.33^{bD} | | | |
| C1 | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| Т | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| R | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| E | <1 | <1 | $0.07\pm0.01^{\text{aA}}$ | 0.23 ± 0.03^{aB} | $1.32\pm0.18^{\text{aC}}$ | $1.80\pm0.24^{\text{aD}}$ | | | |
| TE | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| RE | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| NT | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| NR | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| NE | <1 | <1 | <1 | <1 | <1 | $1.42\pm0.19^{\text{a}}$ | | | |
| NTE | <1 | <1 | <1 | <1 | <1 | <1 | | | |
| NRE | <1 | <1 | <1 | <1 | <1 | <1 | | | |

Note: ^{a,b}The difference between the samples represented with the different letters in the same column is significant (p < .05). ^{A-D}The difference between the samples represented with the different letters in the same row is significant (p < .05).

essential oils (clove, thyme, basil, and cinnamon essential oils) was found to be 1.68 log CFU/g and, among the essential oils used in that study, the essential oil that is most effective against YM growth was reported to be the thyme essential oil (Sharma et al., 2017). In that study, the number of YM on 45th storage day of sausage-containing thyme essential oil was 1.23 log CFU/g. In another study, the effect of rosemary extract on the shelf-life of chicken meatballs was investigated, and it was reported that rosemary was effective on the growth of YM but that effect did not change with the change in the amount of rosemary (Can et al., 2016). In a study, in which chicken meatballs containing 0.4% thymol were stored at 4°C for 12 days, the number of YM at the end of storage period was reported to be 5.13 log CFU/g in control sample and 2.43 log CFU/g in samples containing 0.4% thymol (Can, 2012).

The number of coliform bacteria in the beef Mortadella samples stored at 4°C for 90 days was found to be below the detectable level. Since the psychrophilic microorganisms can develop between 0 and 10°C, it was reported that they are important for fresh or processed meat and meat products stored at cold (Halkman, 2005).

Since the beef Mortadella samples were subjected to the pasteurization process, the total aerobic psychrophilic bacteria (TAPB) values were found to be below the detection threshold on the first days of storage (0th and 7th days; Figure 5). However, on 14th and 30th days, bacterial development was observed in CO, E, and NE; the numbers of TAPB in these groups were found to be 1.49, 1.16, and 0.84 log CFU/g, and the difference between the groups was found to be statistically significant (p < .05). On the 90th day, bacteria development was observed in all the samples, and examining the differences between the groups, it was found that the difference between C0 and E groups was statistically nonsignificant (p > .05), whereas the differences between other groups were found to be significant (p < .05). The highest number of TAPB on the 90th day was found to be 5.69 log CFU/g in CO group, while the lowest number of TAPB was 2.02 log CFU/g in NTE group. The difference between C1 group and groups containing essential oil was found to be statistically non-significant (p > .05). Based on this result, it can be said that the effects of essential oils used in the present study and that of nitrite on psychrophilic bacteria are similar.

Besides that, it was also determined that the numbers of TAPB of T and R groups on the 90th day were higher than those of NT and NR groups. Similarly, the numbers of TAPB in NT and NR groups were found to be lower than that in NTE and NRE groups. When compared with the sole use of essential oils in beef Mortadella samples, the combination with 75 ppm nitrite and 1% red beet extract was found to be more effective in terms of the inhibition of psychrophilic bacteria. The differences between the beef Mortadella samples containing essential oil in terms of TAPB were found to be statistically nonsignificant (p > .05). Thymol and rosemary essential oils showed similar effects on the TAPB in beef Mortadella samples.

Liu et al. (2009) carried out a study on chicken sausages containing different concentrations of rosemary extract and stored at 4°C for 14 days and reported that the numbers of TAPB were 6 log CFU/g in the group containing no rosemary extract, and 6.0, 5.5, and 5.0 log CFU/g in groups containing 500, 1,000, and 1,500 ppm rosemary extract, respectively. In their study, these authors stated that the increase in the concentration of rosemary extract played an effective role in decreasing the number of TAPB. In a different study, it was reported that the numbers of psychrophilic bacteria in experimental chicken sausage samples containing different spice (thyme, rosemary, and oregano) extracts during the storage period have increased (Bayrak, 2011). The number of psychrophilic bacteria in sausages containing thyme extract was lower than in the samples containing other spice extracts (Bayrak, 2011). In that study, it was determined that the number of psychrophilic bacteria increased Journal of Food Processing and Preservation





FIGURE 5 Changes in the number of total aerobic psychrophilic bacteria in beef Mortedalla samples stored at 4°C (log CFU/g). Measurements were taken at 0, 7, 14, 30, 60, and 90th days. Significant differences between means are denoted by superscripts. Letter differences indicate that superscripts were generated on an individual total aerobic psychrophilic bacteria (log CFU/g) basis using modelbased Tukey's HSD

from 2.38 to 12.04 log CFU/g in the control group, from 1.67 to 9.19 log CFU/g in the group containing thyme extract, and from 2.22 to 10.59 log CFU/g in the group containing rosemary extract. These values are higher than those obtained on the 0th and 90th days of storage in the present study. This is thought to be likely because of raw material difference (beef meat—chicken meat) or difference in forms of thyme and rosemary additives (essential oil—extract). In another study on the effects of rosemary extract on storage quality of chicken meat, it was reported that, when compared with the control group, the number of psychrophilic bacteria in samples containing rosemary extract was lower than that in control group, and the lowest number of psychrophilic bacteria on 7th day of storage was found in meatball samples containing 1% rosemary (4.14 log CFU/g; Can et al., 2016).

3.8 | Sensory analysis results

It is clear that, while developing a new meat product or improving an existing meat product, the data are very important, and even if the meat product is acceptable from instrumental or microbiological aspect but not from the sensorial aspect, this product cannot be recommended (Toldrá et al., 2014). It was reported by various authors that the compounds such as aldehyde and ketone emerging due to the lipid oxidation during storage of meat products altered the taste and odor scores (Flores et al., 2004).

Multiple criteria decision-making methods were used in sensory analyses. In the SAW method, sensory scores of the alternatives were directly used, and those values were normalized by using Equation 1. Overall score obtained on all the analysis days was obtained by calculating the sum of the weighted-normalized value of each criterion of the alternatives (Tables 6 and 7). According to the SAW technique, NRE sample had the highest value. It suggests that NRE is the best sample on all the analysis days. At storage period, the best four samples were NRE, NTE, NE, and C1 as ranking the groups with SAW. Comparing general appreciation scores of the samples containing rosemary essential oil and thymol (T–R, TE–RE, NT–NR, NTE–NRE), it was determined that the samples containing rosemary essential oil had higher scores than the samples.

In a study investigating the sensorial effect of tomato powder on the sausage samples, it was reported that the color score and the general acceptability score increased as the tomato powder concentration of samples increased (Eyiler & Oztan, 2011). In a study carried out by Jin et al. (2016), it was reported that the sensorial character, especially the aroma and general appreciation scores, of sausage samples containing thyme and rosemary were negatively affected during the storage period, but no difference was found between the sensorial values of sausage samples at the end of storage period. In a study, in which the authors used red beet powder in sausage samples at different concentrations, Turp et al. (2016) reported that the red beet powder improved the taste of sausage formulation, and the highest sensorial score was obtained with sausage samples containing 4% red beet powder. Similarly, it can be stated that the red beet extract improved the taste score of beef Mortadella samples in the present study. In a different study, it was reported that, among the chicken sausage samples containing thymol at different TABLE 6 Pair-wise comparison matrix of alternatives based on criteria and general scores of alternatives obtained from SAW technique

| | | Normalized | l comparison ma | trix | | Weighted n | ormalized matri | х | |
|----------|-------|------------|-----------------|-------|-------|------------|-----------------|-------|-------|
| | Group | Taste | Appearance | Odor | Color | Taste | Appearance | Odor | Color |
| Oth day | C0 | 0.109 | 0.069 | 0.110 | 0.064 | 0.041 | 0.020 | 0.023 | 0.008 |
| | C1 | 0.158 | 0.148 | 0.137 | 0.183 | 0.059 | 0.043 | 0.029 | 0.023 |
| | Т | 0.104 | 0.085 | 0.110 | 0.082 | 0.039 | 0.025 | 0.023 | 0.010 |
| | R | 0.119 | 0.112 | 0.127 | 0.108 | 0.045 | 0.033 | 0.026 | 0.013 |
| | E | 0.093 | 0.115 | 0.105 | 0.112 | 0.035 | 0.034 | 0.022 | 0.014 |
| | TE | 0.096 | 0.106 | 0.100 | 0.071 | 0.036 | 0.031 | 0.021 | 0.009 |
| | RE | 0.113 | 0.121 | 0.122 | 0.105 | 0.042 | 0.035 | 0.025 | 0.013 |
| | NT | 0.096 | 0.119 | 0.094 | 0.135 | 0.036 | 0.035 | 0.020 | 0.017 |
| | NR | 0.112 | 0.124 | 0.094 | 0.140 | 0.042 | 0.036 | 0.020 | 0.018 |
| | NE | 0.135 | 0.090 | 0.092 | 0.134 | 0.051 | 0.026 | 0.027 | 0.039 |
| | NTE | 0.139 | 0.134 | 0.136 | 0.133 | 0.052 | 0.039 | 0.040 | 0.039 |
| | NRE | 0.151 | 0.141 | 0.138 | 0.148 | 0.057 | 0.041 | 0.040 | 0.043 |
| 90th day | C0 | 0.099 | 0.054 | 0.108 | 0.063 | 0.037 | 0.016 | 0.022 | 0.008 |
| | C1 | 0.146 | 0.155 | 0.147 | 0.175 | 0.055 | 0.045 | 0.031 | 0.022 |
| | Т | 0.096 | 0.081 | 0.113 | 0.085 | 0.036 | 0.024 | 0.024 | 0.011 |
| | R | 0.117 | 0.127 | 0.123 | 0.112 | 0.044 | 0.037 | 0.026 | 0.014 |
| | E | 0.107 | 0.110 | 0.097 | 0.117 | 0.040 | 0.032 | 0.020 | 0.015 |
| | TE | 0.095 | 0.106 | 0.117 | 0.074 | 0.036 | 0.031 | 0.024 | 0.009 |
| | RE | 0.121 | 0.119 | 0.112 | 0.108 | 0.045 | 0.035 | 0.023 | 0.013 |
| | NT | 0.101 | 0.122 | 0.090 | 0.132 | 0.038 | 0.036 | 0.019 | 0.016 |
| | NR | 0.118 | 0.126 | 0.094 | 0.134 | 0.044 | 0.037 | 0.020 | 0.017 |
| | NE | 0.134 | 0.097 | 0.090 | 0.129 | 0.050 | 0.028 | 0.026 | 0.038 |
| | NTE | 0.122 | 0.127 | 0.124 | 0.127 | 0.046 | 0.037 | 0.036 | 0.037 |
| | NRE | 0.144 | 0.143 | 0.131 | 0.146 | 0.054 | 0.042 | 0.038 | 0.043 |

 TABLE 7
 Evaluation scores of the samples on the sensory alternatives with SAW

| | Stroage period (days) | | | | | | | | | | | |
|---------|-----------------------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|
| | 0 | | 7 | | 14 | | 30 | | 60 | | 90 | |
| Ranking | Group | Score | Group | Score | Group | Score | Group | Score | Group | Score | Group | Score |
| 1 | NRE | 0.1808 | NRE | 0.1685 | NRE | 0.1745 | NRE | 0.1808 | NRE | 0.1709 | NRE | 0.1765 |
| 2 | NTE | 0.1696 | C1 | 0.1546 | C1 | 0.1614 | NTE | 0.1619 | NTE | 0.1521 | NTE | 0.1558 |
| 3 | C1 | 0.1541 | NTE | 0.1490 | NE | 0.1429 | C1 | 0.1518 | C1 | 0.1512 | C1 | 0.1524 |
| 4 | NE | 0.1429 | NE | 0.1450 | NTE | 0.1416 | NE | 0.1360 | NE | 0.1429 | NE | 0.1424 |
| 5 | R | 0.1172 | NR | 0.1207 | R | 0.1187 | RE | 0.1298 | R | 0.1252 | R | 0.1205 |
| 6 | RE | 0.1164 | R | 0.1142 | NT | 0.1137 | R | 0.1263 | RE | 0.1184 | NR | 0.1174 |
| 7 | NR | 0.1153 | E | 0.1123 | NR | 0.1099 | NR | 0.1231 | NR | 0.1169 | RE | 0.1167 |
| 8 | NT | 0.1070 | NT | 0.1102 | RE | 0.1095 | NT | 0.1080 | E | 0.1136 | NT | 0.1087 |
| 9 | E | 0.1045 | RE | 0.1074 | Т | 0.1027 | E | 0.1015 | NT | 0.1028 | E | 0.1070 |
| 10 | Т | 0.0972 | TE | 0.0998 | E | 0.1011 | TE | 0.0952 | TE | 0.1028 | TE | 0.1003 |
| 11 | TE | 0.0964 | Т | 0.0964 | TE | 0.1001 | C0 | 0.0823 | Т | 0.0897 | Т | 0.0938 |
| 12 | C0 | 0.0919 | C0 | 0.0844 | C0 | 0.0829 | Т | 0.0820 | C0 | 0.0795 | C0 | 0.0831 |

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TABLE 8 Comparing the samples in terms of quality characteristics

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|-----------------------------------|--------------|----------|---|----------|
| Quality characteristics | Analysis day | C1 | NTE | NRE |
| TBA (mg malonaldehyde) | Oth day | 260.9333 | 2,065.6140 | 265.8700 |
| | 90th day | 201.4143 | 1,767.3983 | 266.0113 |
| DPPH free radical scaventing | Oth day | 73.99 | 72.77 | 75.60 |
| activity (%) | 90th day | 78.10 | 72.67 | 73.55 |
| Total phenolic content (mg gallic | Oth day | 260.9333 | 2,065.6140 | 799.9373 |
| acid/ml) | 90th day | 201.4143 | 1,767.3983 | 616.1277 |
| Instrumental color (a*) | Oth day | 22.75 | 18.69 | 18.55 |
| | 90th day | 23.20 | 17.68 | 17.91 |
| Total aerobic psychrophilic | Oth day | <1 | <1 | <1 |
| bacteria (log CFU/g) | 90th day | 2.15 | 2.02 | 2.14 |
| Sensorial (general appreciation) | Oth day | 4.41 | 3.93 | 4.14 |
| | 90th day | 4 37 | 3.67 | 412 |

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Note: C1: Commercial control; NTE: 75 ppm nitrite + 0.3% thymol + %1 red beet extract; NRE: 75 ppm nitrite + 0.3% Rosemary EO + 1% red beet extract.

concentrations, the sensorial scores of sausages containing thymol were higher than that of the thymol-free control group (Chouliara & Kontominas, 2007).

3.9 | Comparing the quality characteristics of samples

Many parameters are used in determining the quality characteristics of meat products. These parameters include oxidation stability, color stability, sensorial findings, texture profile, and microbiological findings. Given the analyses carried out within the scope of the present study, it can be stated that the parameters found to have statistically significant difference were TBA, DPPH free radical scavenging activity, number of TAPB, redness value measured instrumentally, general sensorial appreciation score, and total phenolic content. The samples yielding the best results in terms of these quality characteristics were C1, NRE, and NTE samples (Table 8). Among the essential oils that can be used by decreasing the nitrite, it can be recommended to use rosemary essential oil and red beet extract in reduced-nitrite and carmine-free meat products because the rosemary essential oil was reported to be more acceptable than thymol from sensorial aspect although thymol has high antimicrobial and antioxidant effects and high phenolic content when compared with rosemary essential oil.

4 | CONCLUSION

Besides the literature, also the results obtained here suggest that the antioxidant, antimicrobial, and coloring effects of nitrite can be achieved by substituting it with essential oils and natural extracts. It is thought that the red beet extract could be a good natural colorant that can be used in meat products instead of carmine. Among the essential oils, it can be recommended to use rosemary essential oil and red beet extract in low-nitrite and carmine-free meat products because the rosemary essential oil was reported to be more acceptable than thymol from sensorial aspect. However, when compared with rosemary essential oil, thymol has high antimicrobial and antioxidant effects and high phenolic content in Mortadella samples. The present study can be further expanded by using these essential oils in different meat products, and these effects can be investigated by using different essential oils as well.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Emre Hastaoglu: Data curation; Funding acquisition; Investigation; Resources; Software; Writing-original draft. **Halil Vural:** Conceptualization; Methodology; Project administration; Supervision; Validation; Writing-review & editing. **Özlem Pelin Can:** Conceptualization; Formal analysis; Funding acquisition; Methodology; Project administration; Supervision; Visualization; Writing-review & editing.

DATA AVAILABILITY STATEMENT

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

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