



Effects of natural nitrite sources from arugula and barberry extract on quality characteristic of heat-treated fermented sausages

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ABSTRACT

This study was designed to compare the effects of natural nitrite sources from the arugula leaves (arugula extract and pre-converted arugula extract) and the use of barberry extract (BE) in heat-treated fermented sausage formulations. Eight different sausages were manufactured as follows: pre-converted arugula extract (PA), arugula extract (A), pre-converted arugula extract + BE (PAB), arugula extract + BE (AB), nitrite + BE (POB), no nitrite + BE (NEB), also positive and negative control groups were prepared with (POC) or without nitrite (NEC). The addition of arugula and barberry extracts reduced the residual nitrite content, in fact PAB had the lowest value with a reduction ratio of 47%. The addition of BE lowered the lipid oxidation compared to other counterparts. The use of arugula extract or pre-converted arugula extract resulted in a lower carbonylation than nitrite free samples. The use of natural extracts lowered the a^* and b^* values compared to control. At the end of the storage, no differences were observed on the overall acceptability of all samples. Combined use of barberry extract with arugula and pre-converted arugula extracts could be used as alternative novel curing agent in heat-treated fermented sausages.

1. Introduction

As consumers become increasingly aware of the relationship between their diets and health, the demand for minimally processed, all-natural food products is rising. Research and development studies have been intensified on natural alternatives to chemical additives to satisfy consumer expectations. Nitrate/nitrite has been added to various meat products to maintain microbial quality, color, and flavor and to prohibit oxidative reactions (Alahakoon, Jayasena, Ramachandra, & Jo, 2015). Due to the apparent wide-spread use of nitrites, consumers are becoming more aware of and concerned about their safety. Nitrite can be transformed into N-nitroso with amines in meat products, known to cause human cancer (Lee, Jo, Yoon, Jeong, & Hur, 2021).

The main dietary nitrate or nitrite sources are drinking water, various vegetables, and processed meat products. Considering this, replacing synthetic nitrite with natural sources in meat products has recently been a hot research topic. Arugula, celery, spinach, parsley, lettuce and red beet have been demonstrated as nitrate-rich vegetables (Ko, Park, & Yoon, 2017; Riel, Boulaaba, Popp, & Klein, 2017). Contrary to synthetic counterparts, the natural nitrate sources are not correlated

with proliferative activities according to the epidemiologic studies (Cantor, Ward, Moore, & Lubin, 2006; Yong et al., 2021). In this regard, various studies were conducted to using nitrate-containing vegetables as natural nitrate sources. In previous studies, radish powder in fermented sausage (Ozaki et al., 2021), celery in cold-smoked sausage links (Eisinaité, Tamkutė, Vinauskienė, & Leskauskaitė, 2020), Chinese cabbage in ground pork sausages (Jeong, Bae, Yoon, Jeong, & Gwak, 2020), radish powder in cured pork products (Bae, Choi, & Jeong, 2020) have been approved as a nitrite substitute.

The use of nitrate provides comparable characteristics only if it could be reduced to nitrite by the actions of bacteria (Alahakoon et al., 2015; Oliveira et al., 2021). Nitrates in vegetables, including celery, turnip, parsley, etc., can pass through the nitrogen cycle in microorganisms, which reduce nitrates to nitrites (Kim et al., 2019a). *Staphylococcus xylosus* and *Staphylococcus carnosus* are microorganisms that possess the nitrogen cycle (Sebranek & Bacus, 2007). The use of converted nitrate to nitrite (pre-converted) by microorganisms with nitrate-reducing activity in vegetables such as pre-converted celery in ham slices (Horsch et al., 2014), pre-converted Swedish chard in pork patties (Shin et al., 2017), pre-converted spinach in cured pork loin (Kim et al., 2019b) and pre-

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converted red beet in meat emulsions (Choi et al., 2017) had thought to have essential superiority compared to the use of nitrate extract. This assessment is associated with a longer time for curing reactions by the necessary conversion of nitrate to nitrite during processing. Therefore, as a part of this study, nitrate in the arugula was utilized as an extract, or nitrate in the arugula was converted to nitrite by the pre-conversion process.

Barberry (*Berberis vulgaris* L.) contains numerous compounds such as vitamin C, malic acid, succinic acid, tannin, berbamine, berberine, berberubin, chlorogenic acid, catechin, gallic acid, and anthocyanins and it has been approved by the FDA to be safe for human consumption (Kermanshahi & Riasi, 2006; Rahimi-Madiseh, Lorigoini, Zamani-Gharaghoshi, & Rafieian-Kopaei, 2017). Phenolic compounds found in barberry fruit have strong antioxidant properties (Jaberi, Kaban, & Kaya, 2020).

The European Food Safety Authority (EFSA) has declared that arugula is an essential source of nitrate, with the highest nitrate concentration up to 6400 ppm (European Food Safety Authority (EFSA), 2008). Notwithstanding this, no study utilized the arugula or pre-converted arugula extract as a natural nitrite source in meat products. Within this framework, this study aimed to investigate the effects of using arugula extract or pre-converted arugula extract and barberry extract as synthetic nitrite alternatives in Turkish-type sausage formulations. This study has two main objectives: 1. To investigate the differences between pre-converted arugula extract and arugula extract in cured sausages. 2. To determine the addition of natural extract sources available that can simultaneously provide all the functions of nitrite.

2. Materials and methods

2.1. Materials

Post-rigor boneless beef (*Musculus semitendinosus*, 72% moisture, 21% protein, 4.3% fat and 1.6% ash, heme pigment level: 168.35 ppm) and beef fat (93.8% lipid, 4.7% moisture, and 0.1% ash) were purchased from a local butcher (Izmir, Turkey).

Lyophilized commercial starter culture (mixture of *Pedococcus acidilactici*, *Lactobacillus Plantarum*, and *Staphylococcus carnosus*) was kindly supplied from Pınar Meat Company (Izmir, Turkey). Nitrate-reducing bacteria *Staphylococcus carnosus* (S-B-61, Bactoform) was kindly donated by Chr. Hansen Inc. (İstanbul, Turkey). The curing agents were provided by Fansada Aroma and Spice Food Products Co. (Ankara, Turkey). Fresh arugula (*Eruca sativa*), spices, and air-dried natural casings (D:36 mm, bovine small intestine) were purchased from a local market in Izmir (Aegean region). Dried barberry fruit (*Berberis vulgaris* L.) was obtained from Tebriz (Iran). Analytical-grade chemicals (Sigma-Aldrich Chemie GmbH, Germany) were used for analysis.

2.2. Preparation of arugula extract (AE) and pre-converted arugula extract (PAE)

Fresh arugula leaves were washed under the running water. The leaves were dried in a tray dryer operated at 30 °C for 24 h (Armfield Limited., Ringwood, Hampshire, England). Leaves were then ground to obtain a fine powder. Arugula powder and distilled water were mixed at a ratio of 1:10 (g/ml) in a flask, and the mixture was placed in a water bath operating at 80 °C for 20 min for the extraction process. To obtain pre-converted arugula extract (PAE), the arugula powder and distilled water mixture (1:10 g/ml) was inoculated (30 °C, 24 h) with 0.025% active nitrate-reducing bacteria (*Staphylococcus carnosus*, S-B-61, Bactoform, Chr. Hansen Inc., Gainesville, FL, USA). Subsequently, both of these mixtures were filtered through Whatman No. 1 filter paper (Sigma-Aldrich, Germany). After filtration, the mixture was concentrated using a rotary evaporator (Heidolph Hei Vap ValG1, Germany) at 50 °C.

Concentrated AE (pH = 5.34, $L^* = 14.24$, $a^* = -0.46 \pm$, $b^* = 13.96$)

(4704.28 ppm nitrate and 23.82 ppm nitrite) and PAE (pH = 5.33, $L^* = 14.24$, $a^* = -0.46 \pm$, $b^* = 13.96$) was used as nitrite sources (514,21 ppm nitrate and 56.38 ppm nitrite). The extracts were stored at 4 °C until their usage within 24 h.

2.3. Preparation of barberry (*Berberis vulgaris*) extract

Barberry extract was prepared according to the method described by Končić et al. (2010). Dried barberry fruits were directly used without any crushing or grinding. Fruits were washed with tap water before being used for the extraction process. Fruit and ethanol were mixed at a ratio of 1:5 (w/v) in a flask and placed into the chamber of a laboratory-grade microwave oven (Sineo Mas-II Plus, Shanghai, China). The extraction was performed at 60 W, at 45 °C for 30 min. Then, the flask was transferred to a shaking water bath at 45 °C, 65 rpm, for 30 min. After the extraction process, the extract was filtered through Whatman No:1 (Sigma-Aldrich, Germany), and the solvent was removed using a rotary evaporator (Heidolph Hei Vap ValG1, Germany) at 50 °C. (Barberry extract: L^* : 19.07 ± 0.65 , a^* : 18.41 ± 0.98 , b^* : 13.40 ± 0.37 , pH: 2.56, ascorbic acid: 429.36 mg/100 g, no toxicity up to 1000 ppm against L929 cell culture (DIN EN ISO 10993-5, 2009)). The bioactive compounds of barberry extract were determined according to Gumus, Moulahoum, Tok, Kocadag Kocazorbaz, and Zihnioglu (2022) with some modifications. Chromatographic separation was performed using a HPLC Agilent 1260 Infinity series (Agilent Technologies, Santa Clara, CA, ABRE) instrument with a Poroshell 120 EC-C18 (3.0 × 150 mm, 2.7 μm particle size) column. The mobile phase system was constructed from a 5 mM ammonium formate in water (A) and methanol (B) using a gradient elution as follows: 0–0.5 min, 10% B; 0.5–5 min, 70% B; 5–7 min, 95% B; 7–10 min, 95% B; 10–15 min, 10% B. The column temperature was kept at 25 °C. The injected sample volume was 10 μL and the flow rate used was 0.5 ml/min. MS analysis was performed using an Agilent 6550 iFunnel equipped with the Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface: drying gas flow, 14.0 L/min; nebulizer pressure, 35 psi; gas drying temperature, 290 °C; sheath gas temperature, 400 °C; sheath gas flow, nitrogen 12 L/min. The scanning range m/z 50 to 1800 was selected. The bioactive compounds of the barberry extract presented in Table 1.

Table 1
Bioactive compounds in barberry extract.

Name	Class	Molecular formula	m/z	Score	Polarity
Trigonelline	Alkaloid	C7H8NO2	138.05	97.12	+
Quercetin	Flavonoid	C15H10O7	303.05	92.12	+
Phenylacetic acid	Phenyl group	C8H8O2	137.06	89.64	+
Palmatine	Alkaloid	C21H22NO4	352.15	96.83	+
Oleic Acid ethyl ester	Fatty acid ester	C20H38O2	311.29	86.96	+
Oleic Acid	Fatty acid	C18H34O2	283.26	86.78	+
Luteolin	Flavonoid	C15H10O6	287.05	100	+
Herbacetin	Flavonoid	C15H10O7	303.05	89.4	+
Genistein	İzoflavon	C15H10O5	271.06	86.51	+
DL-Canadine	Alkaloid	C20H21NO4	340.15	95.92	+
Diosmetin	Flavonoid	C16H12O6	301.07	97.94	+
Chlorogenic Acid	Fenolik asit	C16H18O9	355.10	97.01	+
Apigenin	Flavone	C15H10O5	271.06	100	+
(±)-Naringenin	Flavonoid	C15H12O5	273.07	97.41	+
Quinic acid	Alcohol	C7H12O6	191.06	88.22	-
Quinalizarin	Organic acid	C14H8O6	271.03	97.26	-
Chlorogenic Acid	Fenolic acid	C16H18O9	353.09	85.08	-
Caffeic Acid	Fenolik asit	C9H8O4	179.03	91.67	-
(±)-Naringenin	Flavonoid	C15H12O5	271.06	80.05	-

2.4. Experimental design and preparation of sausages

Eight treatments were produced two times on different days according to Table 2. Each batch was 5 kg (approximately 25 sausages of 200 g baton per repetition) with different sources of nitrite were prepared in two separate replications of the experiment; negative control: no added nitrite (NC), positive control: 150 ppm sodium nitrite (PC), barberry extract (NCB), 150 ppm nitrite+barberry extract (PCB), pre-converted arugula extract (PA), arugula extract (A), pre-converted arugula extract+ barberry extract (PAB), arugula extract+barberry extract (AB). The block diagram of heat-treated fermented sausage can be seen in Fig. 1.

Visible connective tissue and fat were separated from the beef manually by using a sharp, narrow-bladed knife. Lean meat and beef fat were grounded through a 3 mm plate. Minced beef and fat were mixed with the curing ingredients (arugula or pre-converted arugula extracts as 150 ppm nitrite equivalent), spices, lyophilized starter culture mix, and barberry extract (200 ppm gallic acid equivalent). Each prepared sausage dough was stuffed into natural casings derived from bovine small intestines using a hydraulic sausage filling machine (SG-Alpina, Schweiz, Switzerland). Sausage doughs were rested in a conditioning cabinet set to 21 °C and 60% relative humidity (RH) (Daihan Scientific, South Korea) for 3 h. Then, sausage doughs were fermented in the same cabinet where the ambient temperature and RH were 25 °C and 90%, respectively. Fermentation was terminated when the pH value of sausages reached 5.60. After then, fermented samples were placed in an oven (80 °C) (AFOS Mini Kiln, UK) and heat-treated until the center-point temperature reached 68 ± 2 °C. Heat-treated samples were rapidly cooled by spraying water. The sausages were put again into the cabinet and ripened at 18 °C and 72% RH for 24 h. The sausage samples were stored at 4 °C for 3 months to follow up the oxidative changes and residual nitrite during refrigerated storage.

2.5. Determination of total phenolic content and antioxidant capacity of barberry extract

The Folin-Ciocalteu method was used to determine the total phenolic content in barberry extract. The results were expressed as mg gallic acid equivalent (GAE) per gram (Sarkis, Michel, Tessaro, & Marczak, 2014). Various concentrations of gallic acid (50–500 µg/ml) solutions were used to plot a standard curve. The antioxidant capacity of barberry extract was tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described by Grajeda-Iglesias et al. (2016). The results were expressed in terms of micromole Trolox equivalent (TE) per gram by using the Trolox calibration curve.

2.6. Water activity and weight loss

Water activity (a_w) was measured at 25 °C using an a_w measurement device (Testo AG 400, Germany). The device's plastic containers were

filled with about 5 g of the sample before being put in the equipment chamber for equilibration. The result was recorded, and the sample was removed from the chamber. Weight loss was evaluated as a percentage of the initial sample weight during the processing.

2.7. Color parameters

Color parameters of arugula and barberry extracts (L^* -brightness, a^* -redness, b^* -yellowness) were measured with a portable color measurement device (CR-400, Konica Minolta, Japan). Color parameters of the sausage samples were measured from the longitudinal cut surfaces of the sausages using a portable colorimeter (CR-200, Konica Minolta, Japan) equipped with a ten-degree observer angle and D65 illuminant. The color of sausages (final product) was immediately evaluated just after the production. The Chroma (C^*)/Hue angle (h^*), Euclidean distance (ΔE), and redness index (RI) of sausages were calculated according to the American Meat Science Association guidelines (AMSA, 2012).

$$RI = \frac{a^*}{b^*}$$

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$$h^* = \arctan\left(\frac{b^*}{a^*}\right)$$

2.8. Nitrate and nitrite concentration

The amount of nitrate in sausages was determined according to the method proposed by Özdeştan and Üren (2010). A 25 g of the sample was taken and homogenized with 80 ml of distilled water for 5 min. Then 80 ml of hot water (70–80 °C) and 10 ml of 5% (w/v) $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ solution were added and heated 80 °C for 15 min. To clarify the mixture, 5 ml of 23% (w/v) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solution was added and shaken for 15 s, and then 5 ml of 15% (w/v) $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ solution was added and shaken for another 15 s. After cooling, the sample was diluted to 250 ml with distilled water and filtering with Whatman no 40-filter paper. The nitrate in the extract was reduced to nitrite with cadmium acetate solution and zinc powder, and the color of nitrite with Griess reagent was measured spectrophotometrically at 538 nm and the nitrate amount in the sample was expressed as mg/kg.

2.9. Nitroso pigment, total pigment, and curing efficiency

Nitroso pigment and total pigment content of the sausages were determined using the method of Hornsey (1956) during three months of storage. A 10 g of the sausage sample was stirred for 10 min with 40 ml of acetone and 3 ml of water. Following filtering, absorbance at 540 nm was measured against a blank (40 ml acetone +3 ml water). The nitroso pigment concentration (ppm of heme pigment) was calculated as $A_{540} \times 290$. To calculate the total pigment concentration, 10 g of the sausage

Table 2

Formulation of heat-treated fermented sausages.

Groups ¹	Beef (g)	Beef Fat (g)	Salt (g)	Spice (g)	Sugar (g)	Ascorbate (g)	NaNO_2 (g)	AE (g)	PAE (g)	BE (g)
NEC	4000	800	96	180	19.2	1.92	0	0	0	0
POC	4000	800	96	180	19.2	1.92	0.76	0	0	0
PA	4000	800	96	180	19.2	1.92	0	0	75.51	0
A	4000	800	96	180	19.2	1.92	0	60.64	0	0
NEB	4000	800	96	180	19.2	1.92	0	0	0	24.13
POB	4000	800	96	180	19.2	1.92	0.76	0	0	24.13
PAB	4000	800	96	180	19.2	1.92	0	0	75.51	24.13
AB	4000	800	96	180	19.2	1.92	0	60.64	0	24.13

** AE: arugula extract; PAE: pre-converted arugula extract; BE: barberry extract.

¹ NEC: negative control (nitrite-free), POC: positive control (150 ppm nitrite), PA: pre-converted arugula extract, A: Arugula extract, NEB: barberry extract, POB: barberry extract+nitrite PAB:pre-converted arugula extract+barberry extract, AB: arugula extract+barberry extract.

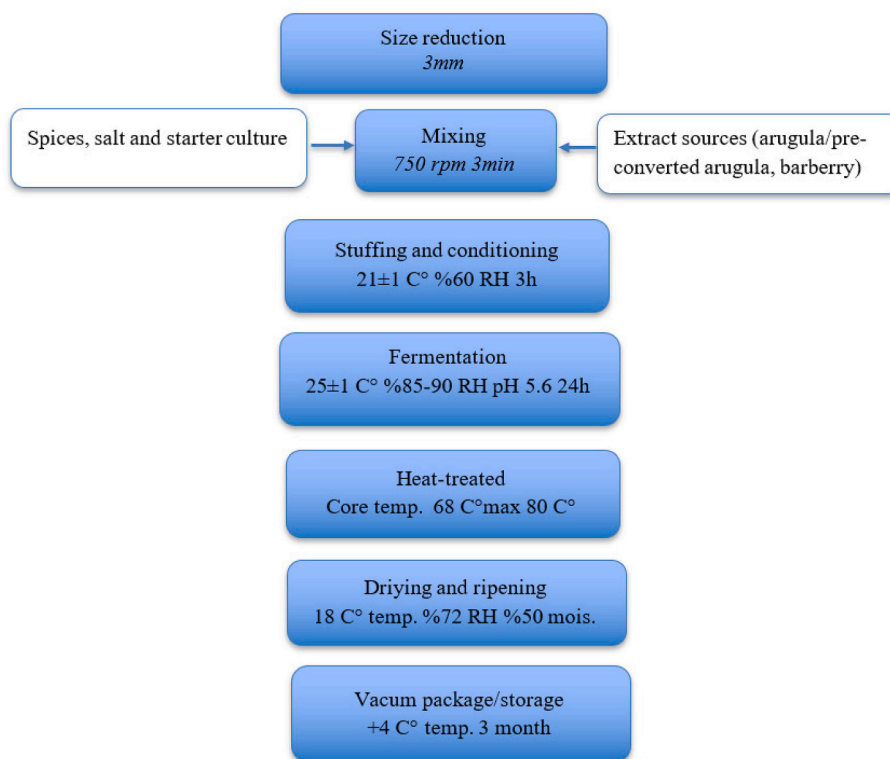


Fig. 1. Preparation of sausages.

was stirred with 40 ml acetone, 2 ml water, and 1 ml HCl and kept in the dark (1 h, room temperature). Then, the solution was filtered. Finally, the absorbance was measured against a blank solution at 640 nm. The total pigment concentration (ppm) was calculated as $A_{640} \times 680$. Then curing efficiency (%) was calculated using the equation:

$$\text{Curing efficiency (\%)} = \frac{\text{Nitroso pigment}}{\text{Total pigment}} \times 100$$

2.10. Lipid oxidation

The method of Witte, Krause, and Bailey (1970) was used to measure the 2-Thiobarbituric Acid Reactive Substances (TBARS) value. Samples were analyzed initially (month 0/final product) and monthly thereafter for 3 months.

2.11. Protein oxidation

The method was used to measure the protein carbonyl products in water extracts using spectroscopy (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008). Protein carbonyl products expressed as nmol carbonyl/mg protein. The method of Sedlak and Lindsay (1968) was used to measure the total sulfhydryl content (nmol -SH/m value). Samples were analyzed initially (month 0/final product) and monthly thereafter for 3 months.

2.12. Sensory evaluation

The sensory panels were carried out in a sensory test laboratory that had partitioned cabinets and individual standard lighting conditions (ISO, 2007). Samples from each formulation were randomly assigned for sensory evaluation. Sensory evaluation was performed using a 9-point hedonic scale (9-like extremely to 1-dislike extremely), and panelists were asked to score the samples in terms of color, cross-sectional appearance, and flavor scored on. In each assessment, there was a

panelist group of 25 people (60%female/40% male, ages 25 to 35). Sausage samples were cut into 0.5 cm thick slices and served warm to panelists with randomly coded digits. Considering the risk of microbial growth, the panelists were not asked to evaluate the samples in terms of flavor during storage.

2.13. Statistical analysis

The entire procedure for the sausage production was replicated twice (two independent batches were produced) on different days and measurements of related traits were conducted in triplicate for each batch. Within one replication, totally eight different sausage formulations (NEC, POC, NEB, POB, PA, PAB, A, and AB) were prepared. For each formulation, three heat-treated fermented sausages per batch were selected for the subsequent analyses. Mean values for measured parameters were calculated and compared by analysis of variance using the SPSS software for windows (SPSS 21.0 for Windows; SPSS Inc. Chicago, IL, USA). Production batches were expressed as a random variable, and each treatment was recognized as a fixed variable. Random variables of sensory analysis were panel day, session count, and the number of panelists. Storage data values were analyzed using two-way ANOVA with treatment and storage time as the main effects. Means were further compared using the Duncan test. Duncan's multiple range test was employed to compare group values with a significance level of $P < 0.05$.

3. Results and discussion

3.1. Total phenolic content and antioxidant capacity of barberry extract

Polyphenols are compounds with antioxidant and antimicrobial activity naturally found in various plants, fruits, and vegetables (Munekata et al., 2021). The total phenolic content and radical DPPH scavenging activity of barberry extract were determined to be 33.16 mg GAE/g and 42.46 $\mu\text{mol TE/g}$, respectively. (Sharifi & Poorakbar, 2015) observed

that barberry extract 41.6 $\mu\text{mol TE/g}$ DPPH value, and Aliakbarlu, Ghiasi, and Bazargani-Gilani (2018) reported that barberry extract had 39.37 mg GAE/g total phenolic content which are similar to our current study findings.

3.2. a_w , pH and total acidity of sausages

The a_w value as a critical shelf-life indicator is affected by the curing ingredients (nitrite, NaCl, i.e.), fermentation and the drying process (Table 3). Initial a_w values were at 0.95–0.98, decreased to 0.93–0.94 after fermentation and to 0.89–0.90 at the end of the ripening (Fig. 2a), which agreed well with our previous data (Öztürk-Kerimoğlu, Kavuşan, Gürel, Çağındı, & Serdaroglu, 2021). No significant differences were found in a_w values among all treatments, and obtained values were below 0.92, the superior limit required to guarantee microbial stability besides pH around 4.9 (Brasil, 2000).

pH and total acidity values are presented in Fig. 2b and Fig. 2c, respectively. Initial pH values were between 5.82 and 5.85, the highest pH was obtained in NEB samples, while the lowest pH value belonged to A samples. The addition of arugula or pre-converted arugula extract did not affect the pH value of the samples. Our results were consistent with the previous study on cured pork loins which showed that spinach juice as a nitrite source did not affect pH values (Kim et al., 2017). On the other hand, different findings compared to our study were reported by Hwang et al. (2018) on pork sausages and by Horsch et al. (2014) on hams when nitrite was replaced with vegetable sources.

pH value was significantly affected by the addition of barberry extract ($P < 0.05$). After the fermentation and heat treatment, sausages with barberry extract had a lower pH value than their counterparts which may be explained by the pH value of the barberry extract (pH: 3.50). These findings align with the previous results that indicate barberry extract could decrease pH values (Aliakbarlu & Mohammadi, 2015; Khaleghi, Kasaai, Khosravi-Darani, & Rezaei, 2016; Jaber et al., 2020; Sayadi, Langroodi, & Pourmohammadi, 2021). pH values of all treatment groups slightly increased after-ripening and ranged from 5.23 to 5.58. The increased pH values may be attributed to proteolysis due to indigenous and microbiological enzyme activity (Mora-Gallego, Guàrdia, Serra, Gou, & Arnau, 2016). After ripening the addition of arugula extract or pre-converted arugula extract caused a decrement in pH values ($P < 0.05$) due to the quite low pH values (AE: 5.34; PAE: 5.33). Since these values were favorable for nitrite degradation, the use of these natural extracts may have an effect on curing reactions.

The titratable acidity (TA) levels in sausage samples during production are reported in Fig. 2c. The main product of carbohydrate fermentation in sausages is lactic acid. The lactic acid level in fermented sausages is the primary reason for the TA increase (Kargozari et al., 2014). The nitrite source did not affect titratable acidity at each evaluation period. However, adding barberry extract increased the TA values of sausage samples. After fermentation, TA levels of sausage doughs ranged from 0.09 to 0.2 lactic acid %, and TA ranged from 0.87 to 0.95 lactic acid % after fermentation, and in the final product, TA was in the range of 0.69–0.78 lactic acid %. In general, the TA was inversely proportional to the pH value. The increments in TA correspond to a decrease

Table 3

The effect of variances on weight loss, a_w and pH values of heat-treated fermented sausages during processing.

Variance source	a_w	pH	Total acidity	Weight Loss
Nitrite source (A)	**	**	n.s	**
Barberry extract (B)	n.s	**	*	**
Processing (P)	**	**	**	**
AxB	**	**	n.s	**
AxP	**	**	n.s	**
BxP	n.s	**	n.s	**
AxBxP	**	**	n.s	**

** $P < 0.01$, * $P < 0.05$, n.s: not significant.

in pH depending on the starter culture metabolism, and the acidity of all sausages increased. The titratable acidity-increasing trend of barberry extract was also obvious at the end of the fermentation ($P < 0.05$). The titratable acidity of sausages ranged between 1.22 and 1.37 lactic acid % when chard powder was added as a nitrate source (Öztürk-Kerimoğlu & Serdaroglu, 2020). Işksal (2021) reported that TA ranged between 0.93% and 1.04 lactic acid % when nitrite in the fermented sausage was reduced or replaced with celery juice and black carrot concentrates.

The highest yield was registered for AB treatment (11.15%) while the lowest yield was found in A treatment (8.82%) (Fig. 2 (d)). All samples had the similar processing yield after the heat treatment ($P > 0.05$). The weight losses were reached to 28.32–33.74% after ripening. The lowest weight losses were observed in NEC, POB, and AB which represents the highest processing yield.

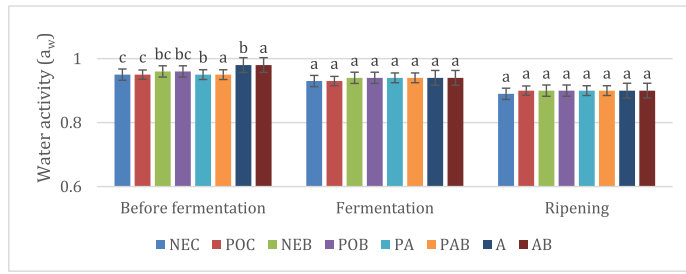
3.3. Color parameters of sausages

Myoglobin is the pigment primarily responsible for the meat color (De Maere et al., 2018). Cured meat color development and stability are due to the combination of nitric oxide with myoglobin (Öztürk Kerimoğlu, Serdaroglu, & Ergezer, 2015). L^* (lightness), a^* (redness), and b^* (yellowness) values of the sausages are shown in Table 4. Nitrite source and arugula extract significantly affected sausage samples' L^* , a^* , and b^* values (Table 6). L^* values changed between 30.96 and 39.33, and AB treatment had the highest L^* value. The addition of barberry extract increased L^* values ($P < 0.05$). POC and POB resulted in higher redness than other treatments. The reducing property of ascorbic acid may be the reason for this effect. It could be said that barberry extract acted as a nitrite reducing agent because its high ascorbic acid content promoted curing reactions. Similar results were also reported by Terns, Milkowski, Rankin, and Sindelar (2011), who observed an increase in curing efficiency by adding cherry powder as a curing accelerator in emulsified cooked sausages.

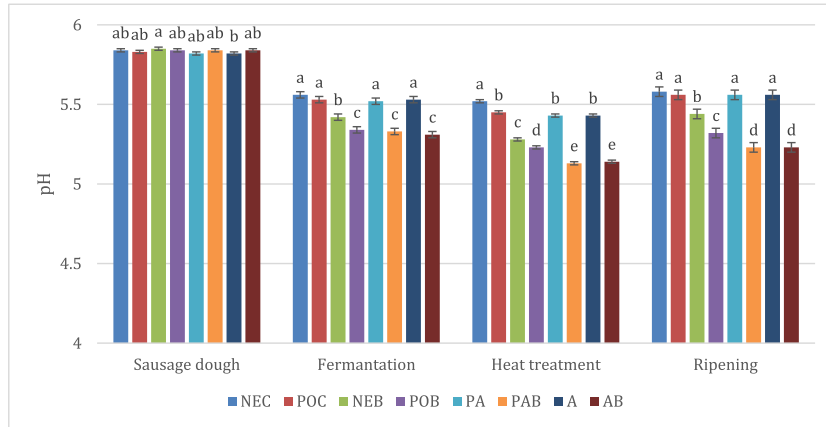
The highest L^* , a^* , and b^* values of heat-treated sausages using radish powder and chitosan as nitrite substitutes were reported as 48.65, 14.70, and 13.97, respectively (Ozaki et al., 2020). Choi et al. (2017) determined that the combined use of fermented red beet extract (*Staphylococcus carnosus*) and ascorbic acid as a nitrite alternative in emulsified meat samples caused an increase in redness. Similarly, the use of barberry extract acted as a reducing agent and accelerated the curing reactions. The b^* values of the samples ranged from 10.81 to 14.95. The POC and NEC treatments revealed the lowest and highest b^* values, respectively.

In the study in which radish powder and thyme essential oil were used as nitrite substitutes, the highest L^* value was 51.7 and a^* and b^* values were found to be highest (11.8 and 8.8) in the groups formulated with radish powder (Ozaki, Dos Santos, et al., 2021). Martínez-Zamora, Peñalver, Ros, and Nieto (2021) evaluated the influence of the combined use of chard and beetroot powders with a starter culture mixture (*Ped-iococcus*, *Staphylococcus xylosus*, and *Staphylococcus carnosus*) in Spanish chorizo. Color analyses indicated that samples produced with the natural nitrite replacers reduced the intensity of the sausages' lightness, redness, and yellowness. The differences between our findings and the some of the published results could be arisen from the natural nitrite source, the addition form or the natural color of the extracts.

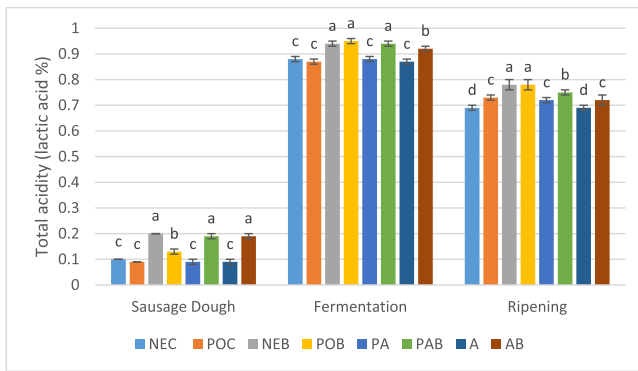
Hue angle index (H°) is the development of color from red to yellow and larger angles imply lower redness; our results show that nitrite source did affect Hue angle values (Table 6). Sausages with no nitrite and pre-converted arugula or arugula extract added had higher hue angles than others ($P < 0.05$), which suggests that these treatments were less red than other counterparts. Also, higher angles were probably sourced by the myoglobin denaturation. The lowest hue angle was registered for positive control groups ($P < 0.05$). Nitrite in the positive control sample was responsible for developing cured meat color. However, using natural nitrite sources was not as effective as nitrite. Besides that, the use of barberry extract contributed redness value (lower hue



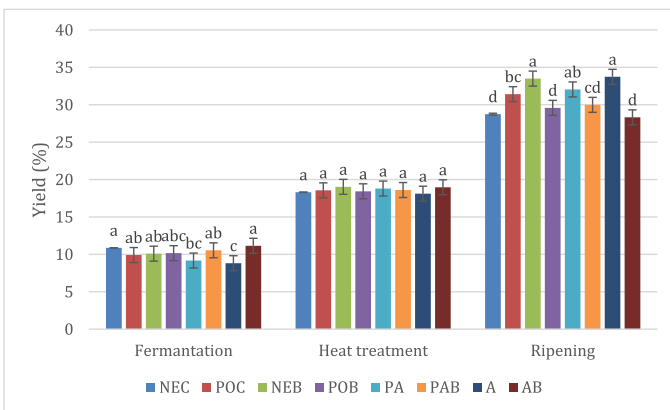
(a)



(b)



(c)



(d)

Fig. 2. a) Water activity (a_w), b) pH, and c) total acidity (lactic acid %) d) yield during sausage process. ^{a-f} Different letters in the same row indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error. ^{x-t} Different letters in the same column indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error. Treatments: NEC: negative control (nitrite-free), POC: positive control (150 ppm nitrite), NEB: barberry extract, POB: barberry extract+nitrite PA: pre-converted arugula extract, A: Arugula extract, PAB: pre-converted arugula extract+barberry extract, AB: arugula extract+barberry extract.

Table 4
Color parameter, nitrosopigment, total pigment and conversion rate of sausages.

Samples ^a	L*	a*	b*	h*(°)	C*	ΔE	RI	Nitroso pigment (ppm)	Total pigment (ppm)	Conversion rate (%)
NEC	34.52 ^c ± 0.52	11.76 ^{bc} ± 0.45	14.95 ^a ± 0.64	51.79 ^a ± 0.33	19.02 ^{ab} ± 1.34	–	0.79 ^c ± 0.01	24.77 ^h ± 0.75	109.36 ^h ± 0.57	22.65 ^g ± 0.88
POC	33.10 ^d ± 0.31	13.74 ^a ± 0.27	10.81 ^c ± 0.27	38.19 ^c ± 0.60	17.49 ^{bc} ± 0.65	5.00 ^{ab} ± 0.07	1.27 ^a ± 0.03	102.64 ^c ± 1.43	152.89 ^d ± 0.09	67.14 ^c ± 0.97
NEB	37.30 ^b ± 0.06	12.28 ^b ± 0.65	12.09 ^{bc} ± 0.42	44.61 ^b ± 0.59	17.23 ^{bc} ± 1.31	4.72 ^{ab} ± 0.05	1.01 ^b ± 0.02	32.81 ^g ± 1.45	118.65 ^g ± 0.59	27.66 ^f ± 1.28
POB	37.73 ^b ± 0.39	13.86 ^a ± 0.46	14.54 ^a ± 0.63	45.64 ^b ± 0.88	20.33 ^a ± 0.84	4.58 ^{ab} ± 0.09	0.98 ^b ± 0.07	157.66 ^a ± 1.55	195.17 ^a ± 0.13	80.78 ^b ± 0.74
PA	30.96 ^e ± 0.84	10.27 ^d ± 0.28	13.25 ^{ab} ± 0.35	52.23 ^a ± 0.72	16.76 ^{bc} ± 0.68	4.09 ^{ab} ± 0.06	0.78 ^c ± 0.03	96.77 ^d ± 0.77	155.93 ^c ± 0.44	62.06 ^d ± 0.66
PAB	37.79 ^b ± 1.15	10.77 ^{cd} ± 0.18	11.77 ^{bc} ± 0.22	47.53 ^b ± 0.99	15.96 ^c ± 0.12	5.94 ^a ± 0.95	0.92 ^b ± 0.03	135.30 ^b ± 1.27	162.00 ^b ± 0.63	83.52 ^a ± 1.06
A	37.85 ^b ± 0.88	10.48 ^{cd} ± 0.03	13.37 ^{ab} ± 0.41	51.89 ^a ± 0.96	16.99 ^{bc} ± 0.53	3.52 ^b ± 0.80	0.79 ^c ± 0.03	42.76 ^f ± 1.07	124.14 ^f ± 0.66	34.44 ^e ± 0.87
AB	39.33 ^a ± 0.75	12.45 ^b ± 0.61	12.70 ^{abc} ± 1.72	45.10 ^b ± 1.56	17.82 ^{bc} ± 2.85	5.17 ^{ab} ± 1.01	1.00 ^b ± 0.09	90.79 ^e ± 0.35	142.32 ^e ± 0.46	63.79 ^d ± 0.32

a-e Different letters in the same column indicate a significant difference ($P < 0.05$). Data were presented as the mean ± standard error.

^a Treatments: NEC: negative control (nitrite-free), POC: positive control (150 ppm nitrite), NEB: barberry extract, POB: barberry extract+nitrite PA: pre-converted arugula extract, A: Arugula extract, PAB: pre-converted arugula extract+barberry extract, AB: arugula extract+barberry extract.

angles), except positive control sample.

Chroma, also known as saturation of meat color, is directly correlated with the myoglobin concentration and status, which stands for the increase in metmyoglobin formation, resulting in chroma values decreasing (Fernández-López, Pérez-Alvarez, Sayas-Barberá, & Aranda-Catalá, 2000; Pérez-Alvarez et al., 1999; Tapp Iii, Yancey, & Apple, 2011). Chroma values ranged between 15.96 (PAB) and 20.33 (POB). The combined use of nitrite and barberry extract showed higher chroma values; on the other hand, using natural nitrite sources, whether PA or A, decreased chroma due to more discoloration in sausage color. The use of barberry extract was ineffective when nitrite was replaced with natural sources ($P > 0.05$).

The total color difference value (ΔE) is the most thriving indicator of the influence of different applications on the meat products color. For a human to perceive the color difference at a glance, the threshold value is considered 2–6 (Larraín, Schaefer, & Reed, 2008; Ramírez-Navas & Rodríguez de Stouvenel, 2012). Total color differences were between 3.52 and 5.94. All samples had similar color differences except for A and PAB samples.

3.4. Nitroso pigment, total pigment, and curing efficiency

The nitroso pigment (NP), total pigment, and pigment conversion rate (curing efficiency) of fermented sausages are summarized in Table 4. The NP content of the samples ranged between 24.77 and 157.66 ppm and was significantly affected by the addition of nitrite sources and barberry extract ($P < 0.05$). As expected, the lowest NP concentration (24.77 ppm) was observed in the negative control samples (without nitrite). The combined use of chemical nitrite and barberry extract resulted in the highest NP formation. Nitrous oxide is responsible for the cured meat color, a nitrosyl nitroso-haemochrome. The probable reason for the NP observed in the nitrite-free (negative control) treatment may be the nitrate that the spices may contain in small amounts. The NP content was higher when pre-converted arugula extract and BE were used as natural nitrite sources rather than 150 ppm commercial nitrite. The pre-converting process raised the NP content of the sausages; NP levels in the PA and PAB treatments were higher than those in the A and AB treatments. The conversion rate of nitrate into nitrite during the fermentation could affect the final nitrite contents in arugula extract and, therefore, the NP content of the sausages.

Total pigment content in the treatments was significantly changed ($P < 0.05$) by the nitrite source and barberry extract addition (Table 4) and ranged from 109.35 to 197.17 ppm. The highest total pigment level was

observed in POB treatment containing 150 ppm chemical nitrite plus barberry extract. The lowest total pigment concentrations were found in NEC and NEB treatments ($P < 0.05$). The long producing period is necessary for nitrate reduction to nitrite, so converted nitrite could be the reason for higher pigment concentration in samples added with pre-converted arugula extract than in samples formulated with arugula extract. This conversion may have been restricted related to fermentation and cooking time. Ozaki, Dos Santos, et al. (2021) also found that nitroso pigment and total pigment tended to decrease by adding radish and beetroot powders as nitrate sources. The general trends in total pigment contents were similar to those observed for the nitrosyl hemochrome contents, suggesting that nitrosyl hemochrome formation may be positively correlated with the total pigment content in meat products. Pre-converted Swiss chard powder in pork patties increased nitroso and total pigment content (Shin et al., 2017). PA treatment gave rise to higher total pigment concentration than POC and A treatments. The addition of barberry extract increased NH pigment and total pigment concentrations of corresponding treatments ($P < 0.05$). Using phenolic acids and flavonoids from natural sources as a reducing agent can potentially increase the nitroso heme pigments (Gao et al., 2022).

The conversion of total pigments to NP pigment indicates the conversion rate and the higher conversion rate implies increased redness (Marches, Cichoski, Zanoelo, & Dariva, 2006; Tambunan, 2008). The conversion rate in our study was between 22.65 and 83.52%. As expected, the lowest conversion rate was found in NRC treatment followed by A treatment. According to Pearson and Tauber (1994), in well-cured meat products the percentage conversion of nitrosoheme pigment to total pigment exceeds 80%. In our study, POB and PAB samples could be counted as well cured in our research. Shin et al. (2017) found the conversion ratio of cooked pork patties added with pre-converted Swiss chard between 9.16 (no nitrite)-82.66% (60 ppm nitrite+3% chard powder), while Ozaki, Munekata, et al. (2021) reported that the conversion ratio was between 10.93 and 18.81% in fermented dry sausage where nitrite was replaced with radish or beetroot powders.

The incorporation of barberry or arugula extracts was effective in lowering the redness index ($P < 0.05$). RI of sausages was in between 0.78 (PA)-1.27 (POC). No nitrite added samples and also A and PA samples had lower RI values compared to positive control samples. The use of barberry extract in the presence of nitrite from arugula increased the RI ($P < 0.05$).

3.5. Lipid oxidation

Lipid oxidation is the reaction that progresses depending on the degree of unsaturated fatty acids with oxidative stress. These reactions are responsible for off-flavor, discoloration, loss of nutritional value, and decreased shelf life of meat products (Cao et al., 2020; Xiong et al., 2020). Nitrites show antioxidant activity and can retard lipid oxidation in meat products. In meat products, 50 ppm nitrite addition could reduce rancidity (Morrissey & Techivangana, 1985; Sebranek, 2009). Fig. 3. represents the effects of nitrite sources and barberry extract on TBARS values during storage. Nitrite source and barberry extract affected TBARS values (Table 6). In the initial storage step (after production), the positive control 150 ppm nitrite-added and POB samples had the lowest TBARS values. In contrast, samples added with arugula extract had the highest TBARS values. The obtained result could be associated with the insufficient conversion of nitrate to nitrite in arugula extract due to the short processing period. Similarly, Krause, Sebranek, Rust, & Mendonca, (2011), observed that incubating curing brines containing vegetable juice powder and starter culture prior to use resulted in comparable lipid oxidation to nitrite added control samples. The antioxidant activity of plant-based extracts depends on the number of polyphenolic compounds. Jeong, Bae, Yoon, Jeong & Gwak, (2020) reported lipid oxidation was more pronounced in cured pork products formulated with cabbage, radish, and spinach powders as nitrite sources.

In contrast to our findings using beetroot and radish powders in dry fermented sausage formulations resulted in lower TBARS values just after the production, this contrast results could be attributed to different processing methods and longer fermentation period (Ozaki et al., 2020). Throughout the storage period, A treatment had the highest TBARS value together with the negative control samples. The lowest TBARS value was achieved in month 3 in PAB treatment ($P < 0.05$). The addition of pre-converted arugula extract resulted in closer results to positive control group that contained nitrite. The use of pre-converted arugula extract could not be able to retard oxidation better than a positive control group, however, the closest group to control was PA ($P < 0.05$). The reason for this situation was the addition of catalase positive *S. carnosus* as a starter culture which is utilized to convert nitrate to nitrite. The starting culture may provide further antioxidant effects because it contains catalase, an important antioxidant enzyme that breaks down hydrogen peroxide. As a result, the starter culture used in natural meat products may have a larger antioxidant effect than starter culture-free conventional meat products. The increasing amount of fermented spinach in cured pork loins lowered the oxidation nevertheless results were higher than control sample (Kim et al., 2017). Also, the

use of fermented spinach, lettuce, celery, and beet was not able to retard oxidation in cooked pork sausages (Hwang et al., 2018). At the end of the storage, all groups that were formulated with barberry extract had the lowest TBARS values except NEB. The phenolic compounds in barberry extract donate hydrogen atoms to the radicals and could prevent the progression of lipid oxidation (Ekici & Sađıç, 2008; Yang, Xie, Jiang, & Wei, 2016). It should be pointed out that oxidation was less pronounced in samples formulated with barberry extract added samples. The antioxidant effect of barberry extract in meat products was reported in different studies (Aliakbarlu & Mohammadi, 2015; Jaber et al., 2020; Sayadi et al., 2021).

3.6. Protein oxidation

Protein oxidation is believed to proceed via a free radical chain reaction similar to that of lipid oxidation although, in the former, higher complexity of the pathways and a larger variety of oxidation products have been reported (Estévez, Morcuende, & Ventanas, 2008). The effects of nitrite source, barberry extract, storage period and their interactions on protein oxidation could be seen in Table 6. Protein oxidation is reflected by the changes in carbonyl content and total sulfhydryl groups. The carbonyl content of fermented sausages is shown in Fig. 4. Initial carbonyl content ranged between 3.82 and 5.18 nmol carbonyl/mg protein.

Carbonyl content of sausages significantly affected by the use of nitrite sources and barberry extract ($P < 0.05$). Due to the absence of nitrite, which is necessary for its antioxidant effect, the NEC treatment had the highest carbonyl content ($P < 0.05$). At the beginning of storage, treatment added with nitrite and barberry extract had the lowest carbonyl content. Barberry extract exhibited an inhibitory effect on protein oxidation when added to sausage formulation ($P < 0.05$). It could be seen that the use of arugula or pre-converted arugula extracts resulted in a higher carbonyl content than POC treatment formulated with 150 ppm nitrite. Carbonyl content increased during the storage in all treatments except NEC. At the end of the third month, the carbonyl content of the sausage samples containing barberry extract was lower than the other samples. Phenolic compounds were reported to have an effect on inhibiting carbonyl formation in various studies (Jongberg, Tørngren, Gunvig, Skibsted, & Lund, 2013; Turgut, Soyer, & Işıkçı, 2016).

The loss of sulfhydryl groups in sausage samples during 3 months of storage is depicted in Fig. 4. In the final product total sulfhydryl content of samples ranged between 36.70 (NEC)-43.86% (POC). The use of natural nitrite sources and barberry extract had a significant effect on

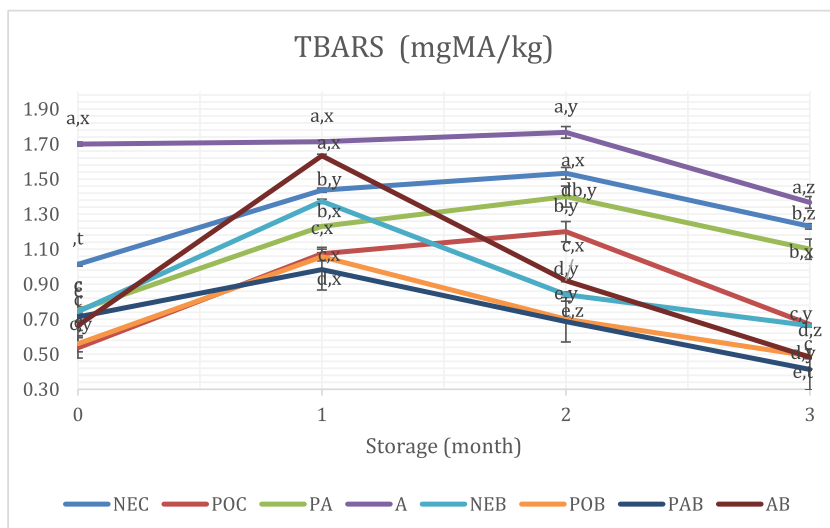


Fig. 3. TBARS of sausages during storage.

^{a-f} Different letters in the same row indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error.

^{x-t} Different letters in the same column indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error.

Treatments: NEC: negative control (nitrite-free), POC: positive control (150 ppm nitrite), NEB: barberry extract, POB: barberry extract+nitrite PA: pre-converted arugula extract, A: Arugula extract, PAB: pre-converted arugula extract+barberry extract, AB: arugula extract+barberry extract.

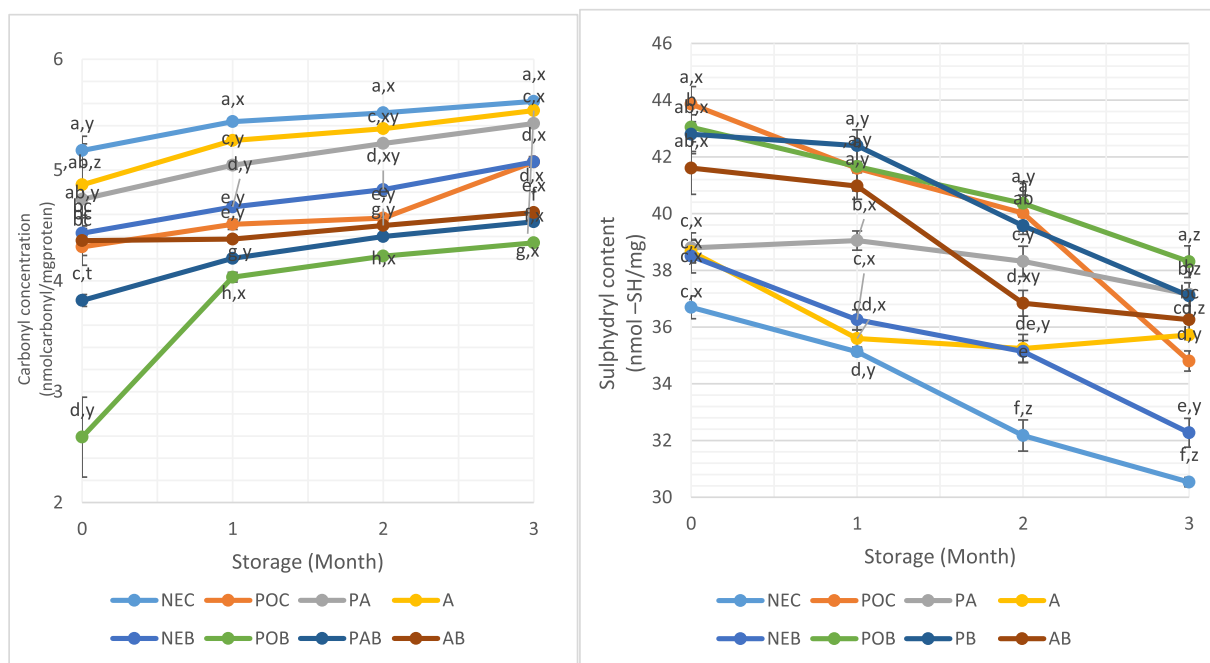


Fig. 4. Protein oxidation in terms of of sausages during storage.

^{a-f} Different letters in the same row indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error.

^{x-z} Different letters in the same column indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error.

Treatments: NEC: negative control (nitrite-free), POC: positive control (150 ppm nitrite), NEB: barberry extract, POB: barberry extract+nitrite PA: pre-converted arugula extract, A: Arugula extract, PAB: pre-converted arugula extract+barberry extract, AB: arugula extract+barberry extract.

total sulfhydryl groups ($P < 0.05$). The lowest total sulfhydryl levels were registered for NEC, NEB, PA, and A samples which elicited that the use of barberry extract has a crucial role in protecting sulfhydryl groups from oxidation. Several studies reported the efficacy of natural antioxidants in meat products. Pomegranate peel extract (Turgut, İşıkçı, & Soyer, 2017), pomegranate fruit juice phenolics (Vaithyanathan, Naveena, Muthukumar, Girish, & Kondaiah, 2011), black currant extract (Jia, Kong, Liu, Diao, & Xia, 2012), rosemary and lemon balm extracts (Lara, Gutierrez, Timón, & Andrés, 2011), clove extract (Zahid et al., 2020), wild thyme by-products extract (Šojić et al., 2020), and purslane extract (Wang, He, Zhang, & Li, 2021) were also delayed the loss of sulfhydryl groups in muscle foods due to their bioactive compounds. Since lipid oxidation products trigger protein oxidation, a possible explanation for high sulfhydryl groups is thought to be lower lipid oxidation in samples formulated with barberry extract. POB and AB had similar initial sulfhydryl groups to positive control groups formulated with sodium nitrite ($P < 0.05$). The loss of sulfhydryl groups was

explicit during the storage due to the progression of oxidation ($P < 0.05$). A decrease in total sulfhydryl groups occurred as a result of the formation of disulfide bonds and tyrosine due to the oxidation of sulfhydryl groups and inter-and intramolecular cross-linking (Zhang, Xiao, & Ahn, 2013). The reduction rate of total sulfhydryl groups was between 4.20 (A) - 20.66 (POC) %. The utilization of barberry extract delayed the protein oxidation compared to corresponding treatments, POB treatment had the highest total sulfhydryl content. However, at the end of the storage, the use of barberry extract was found ineffective in samples where nitrite was replaced with arugula extract ($P > 0.05$). Similar to our results, Martínez-Zamora et al. (2021) indicated that use of chard+beet with rosemary or citrus extract as a natural nitrate source protected the chorizo samples against thiol loss.

3.7. Residual nitrite content

The residual nitrite content of the sausage samples during the storage

Table 5
Residual nitrite content of heat-treated sausages during storage.

Storage (month)	Residual Nitrite Content (mg/kg)							
	NEC	POC	NEB	POB	PA	PAB	A	AB
0	11.27 ^{e,x} ±0.44	22.59 ^{b,x} ±0.41	11.50 ^{ef,x} ±0.61	26.73 ^{a,x} ±0.73	12.44 ^{ef,y} ±0.76	16.19 ^{c,x} ±0.53	13.50 ^{d,y} ±1.13	16.59 ^{c,x} ±0.75
1	10.34 ^{e,y} ±0.60	20.78 ^{b,y,z} ± 0.70	10.65 ^{e,y} ± 0.45	22.81 ^{a,y} ± 1.06	12.81 ^{c,y} ± 0.90	12.64 ^{c,y} ± 0.42	11.60 ^{d,z} ± 0.56	11.73 ^{d,y} ± 0.70
2	10.29 ^{d,y} ±0.73	17.75 ^{b,z,y} ± 0.53	9.32 ^{e,y} ± 0.66	21.53 ^{a,z} ± 0.39	14.51 ^{c,x} ± 0.74	11.40 ^{d,y} ± 0.45	14.46 ^{c,x} ± 0.43	10.61 ^{d,z} ± 0.96
3	9.45 ^{c,z} ±0.73	15.55 ^{a,z} ± 0.62	8.42 ^{c,z} ± 0.58	17.45 ^{a,t} ± 0.74	10.41 ^{b,z} ± 0.53	8.50 ^{c,z} ± 0.75	11.24 ^{b,z} ± 0.57	10.61 ^{b,z} ± 0.54

^{a-f} Different letters in the same row indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error.

^{x-t} Different letters in the same column indicate a significant difference ($P < 0.05$). Data were presented as the mean \pm standard error.

^a Treatments: NEC: negative control (nitrite-free), POC: positive control (150 ppm nitrite), NEB: barberry extract, POB: barberry extract+nitrite PA: pre-converted arugula extract, A: Arugula extract, PAB: pre-converted arugula extract+barberry extract, AB: arugula extract+barberry extract.

could be seen in Table 5. Natural nitrite sources and barberry extract had a significant effect on residual nitrite content (Table 6). In the final product residual content changed between 11.27 and 26.73 ppm. As expected, synthetic nitrite addition resulted in the highest residual nitrite content compared to other treatments at each evaluation period ($P < 0.05$). The residual nitrite content of all treatments was gradually decreased during 3 months of storage ($P < 0.05$). Moreover, residual nitrate contents were decreased with the addition of natural nitrite sources and barberry at the end of the storage PAB and AB. This result could be due to the reaction of nitrite with meat components (mainly lipids, proteins, and myoglobin) and thermal degradation during the cooking process (Honikel, 2008). Other researchers have also indicated similar findings with a lower residual nitrite content being reported in products formulated with alternative natural nitrite sources (Choi, Bae, & Jeong, 2020; Krause, Sebranek, Rust, & Mendonca, 2011; Riel et al., 2017; Sebranek & Bacus, 2007; Shin et al., 2017). Although nitrite free NEC and NEB treatments also had nitrite content, this may be because of the addition of spices that may have contained small amounts of nitrate (Kim et al., 2017). Similarly, Shin et al. (2017) also detected residual nitrite in nitrite-free pork patties. A recent review showed that certain levels of residual nitrite and nitrate in dry-cured meat products could be considered of natural origin such as fresh meat (Iacumin et al., 2019). The pre-converted process was shown to have similar nitrite content with NEC and NEB (no nitrite) treatments during the storage. This finding could be explained by the higher amount of nitrate that was not enough for the bacterial conversion into nitrite. Our result revealed that to obtain low residual nitrite in final products pre-conversion process is necessary. Moreover, the effect of barberry extract was more apparent in samples where nitrite was replaced with natural sources (PAE and AE) because the inclusion of plant-origin ingredients rich in phenolic compounds may contribute to depleting residual nitrite content in meat products (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2009; Wang et al., 2015). Also, antioxidants such as ascorbic acid, can reduce residual nitrite in meat products. (Okatan & Çolak, 2019) indicated that ascorbic acid contents were ranged from 120.36 to 444.35 mg/100 g in *Berberis vulgaris* fruits.

3.8. Sensory analysis

Sensory evaluation results are given in Fig. 5. Positive control groups with or without barberry extract and AB samples had the highest color, texture, and general acceptability scores than other counterparts ($P < 0.05$). Color scores of the samples varied between 5.33 and 8.00. The lowest score was obtained in negative control groups (NEC, NEB). The use of barberry extract increased color scores of positive control or arugula extract added samples due to the natural color pigments in barberry fruit. Similar to our results, Choi et al. (2017) reported that the combination of ascorbic acid+red beet extract resulted in higher color scores in meat emulsions. The higher color scores had been found in the control groups with or without BE and AB treatment. Cross-sectional

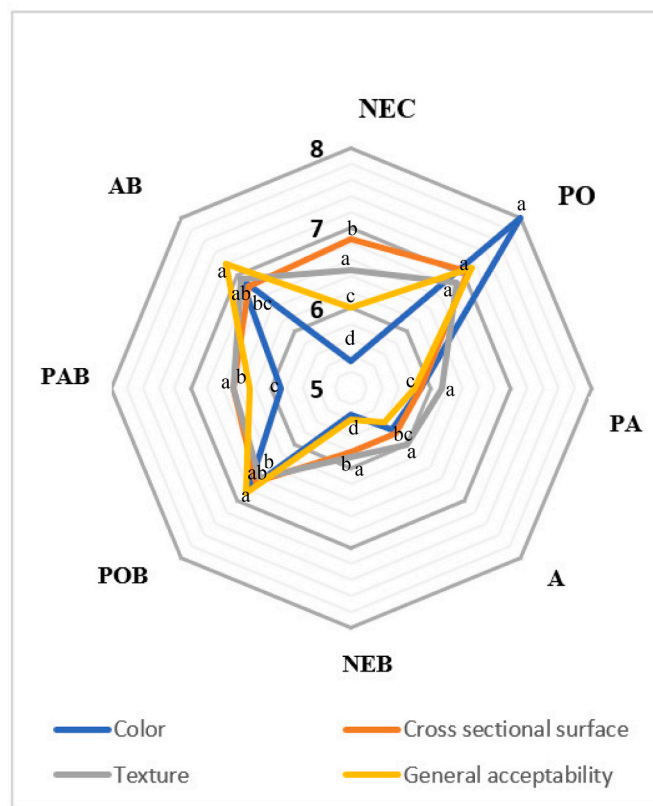


Fig. 5. Color, texture, cross sectional surface, and general acceptability scores of sausages.

Treatments: NEC: negative control (nitrite-free), POC: positive control (150 ppm nitrite), NEB: barberry extract, POB: barberry extract+nitrite PA: pre-converted arugula extract, A: Arugula extract, PAB: pre-converted arugula extract+barberry extract, AB: arugula extract+barberry extract.

area scores of the samples varied between 5.80 and 7.07. No significant differences were observed between the nitrite alternative groups (PAB and AB) and the positive control group (POB). Nitrite alternatives did not cause obvious alteration in the texture of sausages ($P > 0.05$). Ozaki, Dos Santos, et al. (2021) compared the sensory properties of heat-treated sausages in which radish powder and oregano essential oil were used as nitrite substitutes and reported that there was no difference between the samples in terms of overall acceptability, texture, and flavor. Also, curing pork loins with nitrite from Swiss chard did not affect sensory qualities except color (Kim et al., 2019b). The positive control group found the highest general acceptance, and barberry added samples (except for the negative control). From this point of view, it could be said that the addition of barberry had increased the general acceptance

Table 6

The overall effect of nitrite source, barberry extract and storage on some quality parameters of heat-treated fermented sausages.

Variance source	Color							Nitroso-pigment	Total pigment	Conversion rate	TBARS	Carbonyl content	Sulphydryl content	Residual nitrite
	L*	a*	b*	C*	h* (°)	ΔE	RI							
Nitrite source (A)	**	**	n.s	*	**	**	**	**	**	**	**	**	**	**
Barberry extract (B)	**	**	**	n.s	**	*	**	**	**	**	**	**	**	*
Storage (C)	-	-	-	-	-	-	-	-	-	**	**	**	**	**
AxB	**	**	*	*	*	n.s	*	**	**	**	**	n.s	**	**
AxC	-	-	-	-	-	-	-	-	-	-	**	**	**	**
BxC	-	-	-	-	-	-	-	-	-	-	**	n.s	n.s	**
AxBxC	-	-	-	-	-	-	-	-	-	-	**	**	**	**

** $P < 0.01$, * $P < 0.05$, n.s: not significant.

($P < 0.05$). According to Dutcosky (1996), for a product to be considered “well-accepted,” it should have an acceptability index (AI) of $>70\%$. Concerning this target, POC, POB, PAB, and AB samples could be considered as well accepted. The least favored samples were negative control groups and samples added with nitrite alternatives without barberry extract. The addition of fruit and vegetable extract powders and sweet potato powder as nitrite alternatives to emulsified sausages lowered the color and general acceptability scores; however, the addition of paprika+blueberry powder had the opposite effect on sensory product scores (Jin, Choi, Yang, Park and Yim, 2018). Low levels of freeze-dried leek powder resulted in more preferable fermented sausages in terms of appearance, flavor, and overall acceptability (Tsoukalas, Katsanidis, Marantidou, & Bloukas, 2011).

4. Conclusions

Uncured and alternatively cured meat product has gain interest due to increased health concerns. This study reveals that pre-converted arugula can be used to replace synthetic nitrite to produce sausage with similar quality attributes and lower residual nitrite contents for 3 months. The use of pre-converted arugula extract with barberry extract showed the most potential as an alternative to synthetic sodium nitrite, not only maintaining the color development but also inhibiting lipid oxidation of sausages. The use of arugula or pre-converted arugula extract was insufficient to compensate for the antioxidant function of nitrite however, the combined use of barberry extract with these natural nitrate/nitrite sources was a promising strategy to replace nitrite in the formulation. Further studies would be necessary on the inhibitory effects of arugula extract and pre-converted arugula extract on several microorganisms that need to be confirmed in challenge studies.

CRedit authorship contribution statement

Meltem Serdaroglu: Conceptualization, Methodology, Resources, Visualization, Supervision, Project administration. **Hilal Can:** Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Burcu Sarı:** Formal analysis, Writing – original draft. **Hülya Serpil Kavuşan:** Writing – original draft, Methodology, Writing – review & editing, Visualization. **Fatih Mehmet Yılmaz:** Methodology, Investigation, Writing – original draft.

Declaration of Competing Interest

We declare that we have no competing financial interests or personal relationships for the paper entitled “Effects of Natural Nitrite Sources from Arugula and Barberry Extract on Quality Characteristic of Heat-treated Fermented Sausages”.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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References

Alahakoon, A. U., Jayasena, D. D., Ramachandra, S., & Jo, C. (2015). Alternatives to nitrite in processed meat: Up to date. *Trends in Food Science & Technology*, 45, 37–49.

Aliakbarlu, J., Ghiasi, S., & Bazargani-Gilani, B. (2018). Effect of extraction conditions on antioxidant activity of barberry (*Berberis vulgaris* L.) fruit extracts. *In veterinary research forum faculty of. Veterinary Medicine*, 9(4), 361–365.

Aliakbarlu, J., & Mohammadi, S. (2015). Effect of sumac (*Rhus coriaria* L.) and barberry (*Berberis vulgaris* L.) water extracts on microbial growth and chemical changes in ground sheep meat. *Journal of Food Processing and Preservation*, 39, 1859–1866.

AMSA. (2012). *AMSA meat color measurement guidelines*, 61820 pp. 1–135). Champaign, Illinois USA: American Meat Science Association.

Bae, S. M., Choi, J. H., & Jeong, J. Y. (2020). Effects of radish powder concentration and incubation time on the physicochemical characteristics of alternatively cured pork products. *Journal of Animal Science and Technology*, 62, 922–932.

Brasil. (2000). *Instrução Normativa n. 22, de 31 de julho de 2000*.

Cantor, K. P., Ward, M. H., Moore, L., & Lubin, J. (2006). 20 water contaminants. *Cancer epidemiology and prevention*, 382.

Cao, Q., Huang, Y., Zhu, Q. F., Song, M., Xiong, S., Manyande, A., & Du, H. (2020). The mechanism of chlorogenic acid inhibits lipid oxidation: An investigation using multi-spectroscopic methods and molecular docking. *Food Chemistry*, 333, Article 127528.

Choi, J. H., Bae, S. M., & Jeong, J. Y. (2020). Effects of the addition levels of white kimchi powder and acerola juice powder on the qualities of indirectly cured meat products. *Food Science of Animal Resources*, 40, 636–648.

Choi, Y. S., Kim, T. K., Jeon, K. H., Park, J. D., Kim, H. W., Hwang, K. E., & Kim, Y. B. (2017). Effects of pre-converted nitrite from red beet and ascorbic acid on quality characteristics in meat emulsions. *Korean Journal for Food Science of Animal Resources*, 37, 288–296.

De Maere, H., Chollet, S., De Brabanter, J., Michiels, C., Paelinck, H., & Fraeye, I. (2018). Influence of meat source, pH and production time on zinc protoporphyrin IX formation as natural colouring agent in nitrite-free dry fermented sausages. *Meat Science*, 135, 46–53.

DIN EN ISO 10993-5. (2009). *Biologische Beurteilung von Medizinprodukten—Teil 5: Prüfungen auf In-vitro-Zytotoxizität (ISO 10993-5:2009); German Version of EN ISO 10993-5:2009*. Geneva, Switzerland: International Organisation for Standardization.

Dutcosky, S. D. (1996). *Análise sensorial de alimentos*. Curitiba: Champagnat.

Eisainaitė, V., Tamkutė, L., Vinauskienė, R., & Leskauskaitė, D. (2020). Freeze-dried celery as an indirect source of nitrate in cold-smoked sausages: Effect on safety and color formation. *LWT*, 129, Article 109586.

Ekici, L., & Sağdıç, O. (2008). Serbest radikaller ve antioksidan gıdalarla inhibisyonu. *Gıda*, 33, 251–260.

Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., & Heinonen, M. (2008). Fluorescence spectroscopy as a novel approach for the assessment of myofibrillar protein oxidation in oil-in-water emulsions. *Meat Science*, 80(4), 1290–1296.

Estévez, M., Morcuende, D., & Ventanas, S. (2008). Determination of oxidation. In *Handbook Of Processed Meat and Poultry Analysis* (pp. 141–162).

European Food Safety Authority (EFSA). (2008). *Nitrate in Vegetables: Scientific Opinion of the Panel on Contaminants in the Food Chain*. 689 pp. 1–79).

Fernández-López, J., Pérez-Alvarez, J. A., Sayas-Barberá, E., & Aranda-Catalá, V. (2000). Characterization of the different states of myoglobin in pork using color parameters and reflectance ratios. *Journal of Muscle Foods*, 11, 157–167.

Gao, X., Xia, L., Fan, Y., Jin, C., Xiong, G., Hao, X., Fu, C., & Lian, W. (2022). Evaluation of coloration, nitrite residue and antioxidant capacity of the flavonoids, tea polyphenols in cured sausage. *Meat Science*, 192, Article 108877.

Grajeda-Iglesias, C., Salas, E., Barouh, N., Baréa, B., Panya, A., & Figueroa-Espinoza, M. C. (2016). Antioxidant activity of protocathechates evaluated by DPPH, ORAC, and CAT methods. *Food Chemistry*, 194, 749–757.

Gumus, Z. P., Moulahoum, H., Tok, K., Kocadag Kocazorbaz, E., & Zihnioglu, F. (2022). Activity-guided purification and identification of endogenous bioactive peptides from barley sprouts (*Hordeum vulgare* L.) with diabetes treatment potential. *International Journal of Food Science and Technology*, 1–8.

Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, 78, 68–76.

Hornsey, H. C. (1956). The colour of cooked cured pork. I.—Estimation of the nitric oxide-Haem pigments. *Journal of the Science of Food and Agriculture*, 7, 534–540.

Horsch, A. M., Sebraneck, J. G., Dickson, J. S., Niebuhr, S. E., Larson, E. M., Lavieri, N. A., & Wilson, L. A. (2014). The effect of pH and nitrite concentration on the antimicrobial impact of celery juice concentrate compared with conventional sodium nitrite on listeria monocytogenes. *Meat Science*, 96, 400–407.

Hwang, K. E., Kim, T. K., Kim, H. W., Seo, D. H., Kim, Y. B., Jeon, K. H., & Choi, Y. S. (2018). Effect of natural pre-converted nitrite sources on color development in raw and cooked pork sausage. *Asian-Australasian Journal of Animal Sciences*, 31, 1358–1365.

Iacumin, L., Cattaneo, P., Zuccolo, C., Galanetto, S., Acquafredda, A., & Comi, G. (2019). Natural levels of nitrites and nitrates in san Daniele dry cured ham PDO, and in meat, salt and sugna used for its production. *Food Control*, 100, 257–261.

İşıksal, S. (2021). *Sucuk üretiminde bitkisel kaynaklı nitrat kullanımı*. Graduate school of natural and applied sciences (PhD Thesis), Ankara University.

ISO. (2007). *Sensory Analysis-General Guidance for the Design of Test Rooms*. Ref. no. ISO 8589:2007(E). Geneva, Switzerland: International standard 8589.

Jaberi, R., Kaban, G., & Kaya, M. (2020). The effect of barberry (*Berberis vulgaris* L.) extract on the physicochemical properties, sensory characteristics, and volatile compounds of chicken frankfurters. *Journal of Food Processing and Preservation*, 44, Article e14501.

Jeong, J. Y., Bae, S. M., Yoon, J., Jeong, D. H., & Gwak, S. H. (2020). Effect of using vegetable powders as nitrite/nitrate sources on the physicochemical characteristics of cooked pork products. *Food Science of Animal Resources*, 40, 831–843.

Jia, N., Kong, B., Liu, Q., Diao, X., & Xia, X. (2012). Antioxidant activity of black currant (*Ribes nigrum* L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Science*, 91, 533–539.

Jin, S. K., Choi, J. S., Yang, H. S., Park, T. S., & Yim, D. G. (2018). Natural curing agents as nitrite alternatives and their effects on the physicochemical, microbiological

- properties and sensory evaluation of sausages during storage. *Meat Science*, 146, 34–40.
- Jongberg, S., Tørngrøn, M. A., Gunvig, A., Skibsted, L. H., & Lund, M. N. (2013). Effect of green tea or rosemary extract on protein oxidation in Bologna type sausages prepared from oxidatively stressed pork. *Meat Science*, 93, 538–546.
- Kargozari, M., Moini, S., Basti, A. A., Emam-Djomeh, Z., Ghasemlou, M., Martin, I. R., ... Szumny, A. (2014). Development of Turkish dry-fermented sausage (sucuk) reformulated with camel meat and hump fat and evaluation of physicochemical, textural, fatty acid and volatile compound profiles during ripening. *LWT- Food Science and Technology*, 59, 849–858.
- Kerimoğlu, B. Ö., Serdaroglu, F. M., & Ergezer, H. (2015). Et ve Et Ürünlerinde Nitrit-Nitrat; Kullanım Avantajları, Yasal Sınırlamalar ve Güncel Alternatif Yaklaşımlar. *Akademik Gıda*, 13, 257–264.
- Kermanshahi, H., & Riassi, A. (2006). Effect of dietary dried *Berberis vulgaris* fruit and enzyme on some blood parameters of laying hens fed wheat-soybean based diets. *International Journal of Poultry Science*, 5, 89–93.
- Khaloghi, A., Kasaai, R., Khosravi-Darani, K., & Rezaei, K. (2016). Combined use of black barberry (*Berberis crataegina* L.) extract and nitrite in cooked beef sausages during the refrigerated storage. *Journal of Agricultural Science and Technology*, 18(3), 601–614.
- Kim, T. K., Hwang, K. E., Lee, M. A., Paik, H. D., Kim, Y. B., & Choi, Y. S. (2019a). Quality characteristics of pork loin cured with green nitrite source and some organic acids. *Meat Science*, 152, 141–145.
- Kim, T. K., Hwang, K. E., Song, D. H., Ham, Y. K., Kim, Y. B., Paik, H. D., & Choi, Y. S. (2019b). Effects of natural nitrite source from Swiss chard on quality characteristics of cured pork loin. *Asian-Australasian Journal of Animal Sciences*, 32, 1933–1941.
- Kim, T. K., Kim, Y. B., Jeon, K. H., Park, J. D., Sung, J. M., Choi, H. W., ... Choi, Y. S. (2017). Effect of fermented spinach as sources of pre-converted nitrite on color development of cured pork loin. *Korean Journal for Food Science of Animal Resources*, 37(1), 105–113.
- Ko, Y. M., Park, J. H., & Yoon, K. S. (2017). Nitrite formation from vegetable sources and its use as a preservative in cooked sausage. *Journal of the Science of Food and Agriculture*, 97, 1774–1783.
- Končić, M. Z., Kremer, D., Gruz, J., Strnad, M., Biševac, G., Kosalec, I., ... Karlović, K. (2010). Antioxidant and antimicrobial properties of *Moltingia petraea* (Tratt.) Griseb. Flower, leaf and stem infusions. *Food and Chemical Toxicology*, 48, 1537–1542.
- Krause, B. L., Sebranek, J. G., Rust, R. E., & Mendonca, A. (2011). Incubation of curing brines for the production of ready-to-eat, uncured, no-nitrite-or-nitrate-added, ground, cooked and sliced ham. *Meat Science*, 89, 507–513.
- Lara, M. S., Gutierrez, J. I., Timón, M., & Andrés, A. I. (2011). Evaluation of two natural extracts (*Rosmarinus officinalis* L. and *Melissa officinalis* L.) as antioxidants in cooked pork patties packed in MAP. *Meat Science*, 88, 481–488.
- Larrain, R., Schaefer, D., & Reed, J. (2008). Use of digital images to estimate CIE colour coordinates of beef. *Food Research International*, 41, 380–385.
- Lee, S. Y., Jo, C., Yoon, Y., Jeong, J. Y., & Hur, S. J. (2021). Effect on health from consumption of meat and meat products. *Journal of Animal Science and Technology*, 63, 955–976.
- Marches, C. M., Cichoski, A. J., Zanoelo, E. F., & Dariva, C. (2006). Influência das condições de armazenamento sobre os pigmentos cárneos e a cor do salame italiano fatiado. *Food Science and Technology*, 26, 697–704.
- Martínez-Zamora, L., Peñalver, R., Ros, G., & Nieto, G. (2021). Substitution of synthetic nitrates and antioxidants by spices, fruits and vegetables in clean label Spanish chorizo. *Food Research International*, 139, Article 109835.
- Mora-Gallego, H., Guàrdia, M. D., Serra, X., Gou, P., & Arnau, J. (2016). Sensory characterisation and consumer acceptability of potassium chloride and sunflower oil addition in small-caliber non-acid fermented sausages with a reduced content of sodium chloride and fat. *Meat Science*, 112, 9–15.
- Morrissey, P. A., & Techivangana, J. Z. (1985). Antioxidant activities of nitrite and nitrosylmyoglobin in cooked meats. *Meat Science*, 14, 175–190.
- Munekata, P. E., Pateiro, M., Bellucci, E. R. B., Domínguez, R., da Silva Barretto, A. C., & Lorenzo, J. M. (2021). Strategies to increase the shelf life of meat and meat products with phenolic compounds. *In Advances in Food and Nutrition Research*, 98, 171–205.
- Okatan, V., & Çolak, A. M. (2019). Chemical and phytochemicals content of barberry (*Berberis vulgaris* L.) fruit genotypes from Sivasli district of Usak province of western Turkey. *Pakistan Journal of Botany*, 51(1), 165–170.
- Oliveira, W. A., Rodrigues, A. R., Oliveira, F. A., Oliveira, V. S., Laureano-Melo, R., Stutz, E. T., & Guerra, A. F. (2021). Potentially probiotic or postbiotic pre-converted nitrite from celery produced by an axenic culture system with probiotic lactocaseibacilli strain. *Meat Science*, 174, Article 108408.
- Ozaki, M. M., Dos Santos, M., Ribeiro, W. O., de Azambuja Ferreira, N. C., Picone, C. S. F., Domínguez, R., ... Pollonio, M. A. R. (2021). Radish powder and oregano essential oil as nitrite substitutes in fermented cooked sausages. *Food Research International*, 140, Article 109855.
- Ozaki, M. M., Munekata, P. E., Jacinto-Valderrama, R. A., Efraim, P., Pateiro, M., Lorenzo, J. M., & Pollonio, M. A. R. (2021). Beetroot and radish powders as natural nitrite source for fermented dry sausages. *Meat Science*, 171, Article 108275.
- Ozaki, M. M., Munekata, P. E. S., de Lopes, A. S., da Nascimento, M. S., do Pateiro, M., Lorenzo, J. M., & Pollonio, M. A. R. (2020). Using chitosan and radish powder to improve stability of fermented cooked sausages. *Meat Science*, 167, 108–165.
- Özdeştan, Ö., & Üren, A. (2010). *Gıdalarda Nitrat ve Nitrit*. *Akademik Gıda*, 8, 35–43.
- Öztürk-Kerimoğlu, B., Kavuşan, H. S., Gürel, D. B., Çağındı, Ö., & Serdaroglu, M. (2021). Cold-set or hot-set emulsion gels consisted of a healthy oil blend to replace beef fat in heat-treated fermented sausages. *Meat Science*, 176, Article 108461.
- Öztürk-Kerimoğlu, B., & Serdaroglu, M. (2020). Residual nitrite content of heat-treated sucuk as affected by chard powder incorporation and processing. *Gıda/The Journal of Food*, 45, 825–835.
- Pearson, A. M., & Tauber, F. W. (1994). *In processed meats (2nd ed.) the AVI*; Westport, CT, USA: 1984. *Analytical methods* (pp. 351–388).
- Pérez-Alvarez, J. A., Sayas-Barberá, M. E., Fernández-López, J., Gago-Gago, M. A., Pagán-Moreno, M. J., & Aranda-Catalá, V. (1999). Chemical and color characteristics of Spanish dry-cured ham at the end of the aging process. *Journal of Muscle Foods*, 10, 195–201.
- Rahimi-Madiseh, M., Lorigoini, Z., Zamani-Gharaghoshi, H., & Rafeian-Kopaei, M. (2017). *Berberis vulgaris*: Specifications and traditional uses. *Iranian Journal of Basic Medical Sciences*, 20, 569–587.
- Ramirez-Navas, J. S., & Rodriguez de Stouvenel, A. (2012). Characterization of Colombian quesoillo cheese by spectrophotometry. *Vitae*, 19, 178–185.
- Riel, G., Boulaaba, A., Popp, J., & Klein, G. (2017). Effects of parsley extract powder as an alternative for the direct addition of sodium nitrite in the production of mortadella-type sausages—impact on microbiological, physicochemical and sensory aspects. *Meat Science*, 131, 166–175.
- Sarkis, J. R., Michel, I., Tessaro, I. C., & Marczak, L. D. F. (2014). Optimization of phenolics extraction from sesame seed cake. *Separation and Purification Technology*, 122, 506–514.
- Sayadi, M., Langroodi, A. M., & Pourmohammadi, K. (2021). Combined effects of chitosan coating incorporated with *Berberis vulgaris* extract and *Mentha pulegium* essential oil and MAP in the shelf life of Turkey meat. *Journal of Food Measurement and Characterization*, 15, 5159–5169.
- Sebranek, J. G. (2009). Basic curing ingredients. In Tarte (Ed.), *In ingredients in Meat Products* (pp. 1–23). New York, NY: Springer.
- Sebranek, J. G., & Bacus, J. N. (2007). Cured meat products without direct addition of nitrate or nitrite: What are the issues? *Meat Science*, 77, 136–147.
- Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, 25, 192–205.
- Sharifi, F., & Poorakbar, L. (2015). The survey of antioxidant properties of phenolic compounds in fresh and dry hybrid Barberry fruits (*Berberis integerrima* × *vulgaris*). *Cumhuriyet Üniversitesi Fen Edebiyat Fakültesi Fen. Bilimleri Dergisi*, 36(3), 1609–1617.
- Shin, D. M., Hwang, K. E., Lee, C. W., Kim, T. K., Park, Y. S., & Han, S. G. (2017). Effect of Swiss chard (*Beta vulgaris* var. *cicla*) as nitrite replacement on color stability and shelf-life of cooked pork patties during refrigerated storage. *Korean Journal for Food Science of Animal Resources*, 37, 418–428.
- Šojić, B., Tomović, V., Kocić-Tanackov, S., Kovačević, D. B., Putnik, P., Mrkonjić, Ž., ... Pavlič, B. (2020). Supercritical extracts of wild thyme (*Thymus serpyllum* L.) by-product as natural antioxidants in ground pork patties. *LWT*, 130, Article 109661.
- Tambunan, R. D. (2008). Determination of cured meat pigments on three cured beef muscles. In *Seminar Nasional Teknologi Peternakan dan Veteriner, Bogor*.
- Tapp Iii, W. N., Yancey, J. W. S., & Apple, J. K. (2011). How is the instrumental color of meat measured? *Meat Science*, 89, 1–5.
- Terns, M. J., Milkowski, A. L., Rankin, S. A., & Sindelar, J. J. (2011). Determining the impact of varying levels of cherry powder and starter culture on quality and sensory attributes of indirectly cured, emulsified cooked sausages. *Meat Science*, 88, 311–318.
- Tsoukalas, D. S., Katsanidis, E., Marantidou, S., & Bloukas, J. G. (2011). Effect of freeze-dried leek powder (FDLP) and nitrite level on processing and quality characteristics of fermented sausages. *Meat Science*, 87, 140–145.
- Turgut, S. S., İşıkçı, F., & Soyer, A. (2017). Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. *Meat Science*, 129, 111–119.
- Turgut, S. S., Soyer, A., & İşıkçı, F. (2016). Effect of pomegranate peel extract on lipid and protein oxidation in beef meatballs during refrigerated storage. *Meat Science*, 116, 126–132.
- Vaithiyathan, S., Naveena, B. M., Muthukumar, M., Girish, P. S., & Kondaiah, N. (2011). Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4 C). *Meat Science*, 88, 409–414.
- Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2009). Effect of adding citrus waste water, thyme and oregano essential oil on the chemical, physical and sensory characteristics of a bologna sausage. *Innovative Food Science & Emerging Technologies*, 10, 655–660.
- Wang, Y., Li, F., Zhuang, H., Chen, X., Li, L., Qiao, W., & Zhang, J. (2015). Effects of plant polyphenols and α -tocopherol on lipid oxidation, residual nitrites, biogenic amines, and N-nitrosamines formation during ripening and storage of dry-cured bacon. *LWT- Food Science and Technology*, 60, 199–206.
- Wang, Z., He, Z., Zhang, D., & Li, H. (2021). Antioxidant activity of purslane extract and its inhibitory effect on the lipid and protein oxidation of rabbit meat patties during chilled storage. *Journal of the Science of Food and Agriculture*, 101(5), 1953–1962.
- Witte, V. C., Krause, G. F., & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science*, 35, 582–585.
- Xiong, Q., Zhang, M., Wang, T., Wang, D., Sun, C., Bian, H., & Xu, W. (2020). Lipid oxidation induced by heating in chicken meat and the relationship with oxidants and antioxidant enzymes activities. *Poultry Science*, 99, 1761–1767.
- Yang, D., Xie, H., Jiang, Y., & Wei, X. (2016). Phenolics from strawberry cv. Falandi and their antioxidant and α -glucosidase inhibitory activities. *Food Chemistry*, 194, 857–863.

- Yong, H. I., Kim, T. K., Choi, H. D., Jang, H. W., Jung, S., & Choi, Y. S. (2021). Clean label meat technology: Pre-converted nitrite as a natural curing. *Food Science of Animal Resources*, *41*, 173–184.
- Zahid, M. A., Choi, J. Y., Seo, J. K., Parvin, R., Ko, J., & Yang, H. S. (2020). Effects of clove extract on oxidative stability and sensory attributes in cooked beef patties at refrigerated storage. *Meat Science*, *161*, Article 107972.

- Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition*, *53*, 1191–1201.