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Combined Use of Black Barberry (*Berberis crataegina* L.) Extract and Nitrite in Cooked Beef Sausages during the Refrigerated Storage

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ABSTRACT

Effects of combined use of black barberry (*Berberis crataegina* L.) extract and sodium nitrite on the quality and shelf life of cooked beef sausages were investigated. Different concentrations of the extract (30, 60 and 90 mg kg^{-1}) in combination with nitrite (30, 60 and 90 mg kg^{-1}) were added to sausage formulations.. Total viable counts, pH, proximate analysis, residual nitrite level, lipid oxidation, color and sensory data were studied against the blank and control samples during the storage for 30 days at 4°C. A gradual decrease in the nitrite level was observed during the storage for all samples studied. Samples using the extract from this study showed similar redness but lower lightness when compared to the control sausage sample with 120 mg kg^{-1} sodium nitrite. Sensory evaluation of the samples indicated similar results to those of the control. Accordingly, there is a potential benefit for partial replacement of sodium nitrite with barberry extract in the cured meat products.

Keyword: Antibacterial activity, Antioxidant, Refrigeration, Shelf-life, Sensory.

INTRODUCTION

Curing agents including $NaNO_2$ and KNO_2 are widely used in meat products to develop the characteristic flavor and the red meat color, to inhibit growth and toxin production of poisoning anaerobic microorganisms such as *Clostridium botulinum* and to delay spoilage and oxidative rancidity (Pourazrang *et al*., 2002; Zanardi *et al*., 2002).

According to FDA legislation for additives and preservatives in meat products, concentration of sodium or potassium nitrite should not exceed 120 mg kg^{-1} in the final product. Intake of high amount of nitrite presents human health risks including metamyoglobin production as well as vasodilator and allergenic effects (Cammack *et al*., 1999). Nitrous acid produced from $NaNO_2$ may react with secondary amines and amino acids of meat products and form N-nitroso compounds, e.g. nitrosamines, that show mutagenic, toxic, nephrotoxic, neurotoxic, and carcinogenic effects (Karl-Otto, 2008; Rywotycki, 2002). Therefore, there has been a growing interest to reduce or eliminate nitrite in the food products. Due to the antioxidant, and antimicrobial properties of many herbs, plants, fruits, and vegetables, they are excellent candidates for preservatives (Dowlati *et al*., 2015; Maaroufi *et al*., 2015; Mazidi *et*

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al., 2012; Mousavinejad *et al*., 2015). Many natural ingredients such as citrus fruits by-products (Fernandez-Lopez *et al*., 2004), green tea (Bozkurt, 2006), grape seed (Brannan, 2009; Lau and King, 2003), rosemary and oregano (Hernández-Hernández *et al*., 2009) have been considered as preservatives. In the past few years, various plant materials containing natural preservatives such as phenolic compounds have demonstrated antioxidant and antimicrobial activities in the sausage (Coutinho de Oliveira *et al*., 2012; Sallam, *et al*., 2004; Viuda-Martos *et al*., 2010). Black barberry *(Berberis crataegina)* belongs to the family Berberidaceae. This plant is used worldwide in traditional medicine (DeveliIsikh and Yilmaz, 2011). Fruits of *Berberis sp.* are rich in vitamin C, anthocyanins and other phenolic compounds (Wallace and Giusti, 2008; Akbulut *et al*., 2009; Parichehr and Golkho, 2009; Gulsoy and Ozkan, 2011). *Berberis sp.* extracts have good antioxidant and antimicrobial activities (Mollaei *et al*., 2010; Gulsoy and Ozkan, 2011). This is due to the anthocyanins and other phenolic compounds present in such extracts (Konczak and Zhang, 2004). To the best of the authors' knowledge, black barberry has not been reported in any food products as additives. This study is the first communication on the combined use of barberry extract and sodium nitrite in a cured meat product. We aimed to investigate the effects of combined use of black barberry (*Berberis crataegina*) extract and sodium nitrite on the quality and shelf-life of cooked beef sausages.

MATERIALS AND METHODS

Plant Material and the Extraction Procedure

Black barberry was purchased from a local market and dried in a dark place at room temperature. Dried fruits were ground using a dry grinder and stored in sealed multi-lamellar bags at -20ºC. The sample was mixed with enough amount of petroleum ether (40-60 grade) and stirred at 250 rpm in an orbital shaker for 12 hours at room temperature. After solvent removal, the sample was dried using medium air flow. The samples were then suspended in ethanol (0.1%, v/v) and stirred at 150 rpm in orbital shaker for 24 hours at room temperature. After filtration (using Whatman No.1 filter paper), the residual solvent was removed at 40^{o}C using a rotary evaporator. Collected dried extract was stored at 4°C in a dark place until use (Mollaei *et al*., 2010).

Preparation of Beef Sausage

Eleven sausage samples (S1 to S9, control and blank) were prepared according to the formulations specified in Table 1. The sausage samples manufactured in 5-kg batches according to the formulations: beef (3.1 kg), ice (0.92 kg), soybean oil (0.7 kg), NaCl (0.08 kg), starch (0.14 kg), soybean isolate (0.09 kg), $Na_5P_3O_{10}$ (0.02 kg), ascorbic acid (0.01 kg), and spice blend (0.05 kg, Bratwurst, A.C. Legg, USA). The cooked beef sausages were made in the

Table 1. Sample identities and the levels of barberry extract and sodium nitrite added to the sausage samples in this study (3 replicates).

Samples	Nitrite level (mg kg^{-1})	Barberry extract (mg kg^{-1})
Control	120	-
Blank	-	-
S1	30	30
S2	30	60
S3	30	90
S4	60	30
S5	60	60
S6	60	90
S7	90	30
S8	90	60
S9	90	90

R and D department of Solico Meat Product Company (Tehran, Iran). Beef, salt, $Na_5P_3O_{10}$ and $NaNO_2$ were placed in a cutter (Seydelmann, Aalen, Germany) and mixed for approximately 1 minute. Fifty percent of the ice and spice blends were then added and mixed at a high speed. After that, the speed of the cutter was reduced and soybean oil was added and mixed for 5 minutes. The remaining 50% of the ice, starch, ascorbic acid and extract (at different levels according to Table 1) were added and mixed. Each sample was prepared in a polyamide package and cooked at 75ºC for 1 hour. Finally, the samples were cooled with water and stored at 4ºC for 30 days.

Compositional Analysis of Sausage Samples

Fat, protein, moisture, and ash contents of sausage samples were determined in day 1, according to Association of Official Analytical Chemist method (AOAC, 2002a, b, c, d). In order to measure the pH values, 10.0 g of ground samples were mixed with 90 mL distilled water for 1 minute. The measurement was then performed by using a digital pH-meter (Model Glp22, Crison, Barcelona, Spain).

Nitrite Content

Nitrite contents of the sausage samples were determined using a reference method (ISO No 2918/75, ISO, 1975). Briefly, 10 grams of ground sausage samples were mixed with 5 mL borax and 100 mL warm (80ºC) distilled water. After cooling the samples to the room temperature, 2 mL of potassium ferrocyanide trihydrate solution (10.6%) and 2 mL of zinc acetate dihydrate solution (2.2 g zinc acetate dihydrate and 3 mL acetic acid in 100 mL distilled water) were added to the mixture and left at room temperature for 30 minutes. The mixture was filtered with Watman no. 1 filter paper and then 10 mL of the filtrate was mixed with 10 mL of sulfanilamide solution (2.0 g sulfanilamide and 100 mg sulphuric acid in 1,000 mL distilled water) and 6 mL of HCl 45%. After 5 minutes, 2 mL of N-(1-α-naphthyl) ethylenediamine dihydrochloride solution 0.1% was added and placed in a dark room for 10 minutes. Absorbance measurements were then made at 538 nm using a spectrophotometer (2100 spectrophotometer, Unico, China). Nitrite concentrations were determined according to the equation $Y= 9.49X+ 0.096$ with Y representing the nitrite concentration (mg kg^{-1}), and X representing the absorbance values at 538 nm.

Microbial Analysis

After peeling the sausage samples, 10 g was cut aseptically into slices in a sterile container and homogenized with 90 mL of sterile 0.1% peptone water for 2 minutes in a stomacher (Lab blender 400, Seward Medical, London, UK). Then, appropriate decimal dilutions were made from the resultant solution using first diluents (10^{-1}) and plated in duplicate for the following measurements. Total Viable Counts (TVC) were enumerated by the pour-plate method using plate count agar (Merck, Darmstadt, Germany). Plates of TVC were incubated at 30^{o}C for 3 days (ISO 4833: 2003). *C. perfringens* enumeration was performed using an anaerobic culture in sulfite polymyxin sulfadiazine agar (SDS agar, Merck, Darmstadt, Germany) (Yetim *et al*., 2006).

Determination of Antioxidant Activities

The antioxidant activities of the samples were determined using 2, 2'-DiPhenyl-1-PicrylHydrazyl (DPPH) reagent according to the procedure described by Viuda-Martos *et al*. (2009). Samples (0.1 g each) were mixed vigorously with ethanol (4 mL) for 2 minutes. Then, 2 mL of 250 µmol L^{-1} DPPH solution was mixed with 2 mL of the extract

solution for 1 min and placed in a dark room for 30 minutes. Absorbance measurements were taken at 517 nm. Inhibition activities of the samples against DPPH free radical was then determined according to the following expression:

Inhibition ratio (%)= $[(A_{blank}-A_{sample})/A_{blank}]\times 100$

Where, A_{blank} is the absorbance of the control reaction (containing all the reagents except the test sample) and A_{sample} is the absorbance measured for the test sample. The ThioBarbituric Acid Reactive Substances (TBARS) of the samples were measured after 2, 9, 16, 23 and 30 days of storage according to the method reported by Pfalzgraf *et al.* (1995). Ten grams of the sample was homogenized with 20 mL of 10% (w/v) trichloroacetic acid using an ultra-Turrax (90 seconds, 20,000 rpm). The homogenate was centrifuged and the supernatant was filtrated through a paper filter (Watman NO. 1). Two mL of the filtrate was mixed with 2 mL of the TBA (2-thiobarbituric acid) reagent (300 mg TBA in 100 mL water). The mixture was heated on a water bath at 97ºC for 20 minutes. After cooling the mixture to the ambient temperature, the absorption was measured at 532 nm. TBARS values were expressed as mg of malondialdehyde per kg sample using 1, 1, 3, 3-tetraethoxypropane as standard.

Color Measurements

After microbiological and chemical composition measurements, the samples with lowest TBARS value and also microbial content were selected for determining the color stability during the 30 days of storage at 4ºC. Color determination was made by using a Hunter-Lab Color-flex colorimeter (Hunter Associated Lab, Inc., Reston, VA) against a white standard. Each sample was measured at four different locations on the surface and an average score was reported. CIE Lab coordinates were reported as Lightness (L*), redness (a*) and yellowness (b*).

Sensory Evaluation

The samples with the best results from microbiological and chemical attributes were selected for the sensory analysis. Sensory evaluation (flavor, odor and overall acceptability) of the samples were performed using a nine-point hedonic scale (9= Like extremely; 8= Like very much; 7= Like moderately; 6= Like slightly; 5= Neither like nor dislike; 4= Dislike slightly; 3= Dislike moderately; 2= Dislike very much, and 1=Dislike extremely) in day 1. During the sensory evaluation, samples were shared in the sensory room of Sari Agricultural University's laboratory where no foreign smell was present. The product was defined as unacceptable (a score < 5). The panel consisted of 15 trained individuals in the Food Science and Technology Department of Sari Agricultural University.

Statistical Analysis

All experiments were carried out in triplicate and the data were analyzed by SPSS 18 for Windows to assess the significance of the treatments. Analysis Of Variance (One way ANOVA) was conducted and means were compared using Duncan's multiple-range test applying a 95% confidence level.

RESULTS AND DISCUSSION

Compositional Properties of the Cooked Beef Sausages

Protein, fat, ash, and moisture contents of all samples on the first day after production are shown in Table 2. Statistical analysis of the data did not show any significant differences among the samples with the different levels of added nitrite and black

Table 2. Proximate analysis of cooked beef sausages (3 replicates) during the 30 days of storage at 4ºC.

Samples[a]	Composition (%, w/w)			
	Protein	Fat	Moisture	Ash[b]
S1	13.3 ± 0.4	23.5 ± 1.1	61.1 ± 0.0	2.2 ± 0.0^{e}
S2	13.3 ± 0.8	22.8 ± 1.8	61.5 ± 0.0	2.4 ± 0.0^{abc}
S3	13.4 ± 0.2	23.5 ± 0.4	60.6 ± 0.4	2.5 ± 0.0^{a}
S4	13.1 ± 1.1	23.3 ± 0.4	61.3 ± 2.2	2.3 ± 0.0^{ed}
S5	13.4 ± 0.5	23.5 ± 1.8	60.8 ± 1.2	2.3 ± 0.0^{ed}
S6	13.8 ± 0.1	23.6 ± 2.2	60.2 ± 0.8	2.4 ± 0.0^{abc}
S7	13.3 ± 0.9	22.4 ± 1.5	60.9 ± 1.3	2.3 ± 0.0^{bcd}
S8	13.4 ± 1.2	23.8 ± 0.5	60.4 ± 2.2	2.4 ± 0.0^{abc}
S9	13.3 ± 0.4	24.0 ± 0.4	60.3 ± 0.5	2.4 ± 0.0^{abc}
Control	13.4 ± 0.7	24.1 ± 0.8	60.2 ± 1.3	2.3 ± 0.1^{bcd}
Blank	12.9 ± 1.4	23.9 ± 2.4	61.1 ± 0.4	2.1 ± 0.1^{f}

[a] Sample symbols are defined in Table 1. [b] In each column, means identified with different letters are significantly different (P< 0.05).

barberry extract but blank had lower ash level than the other samples (P< 0.05).

Changes in the Rsidual Nitrite Content

Measured nitrite contents of the samples of this study during the 30 days of storage are shown in Table 3. Higher concentrations of added nitrite resulted in higher amounts of residual nitrite in the product. These results are in agreement with the findings of Perez-Rodriguez *et al.* (1996) and Fernandez-Lopez (2007) in cured sausages. The nitrite contents of the samples decreased gradually during the 30 days of storage due to the conversion of nitrites into nitric oxide. Hustad *et al*. (1973) reported significant impacts of both storage time and temperature on the nitrite concentration. Since storage temperature was held constant in this study, storage time can be considered as the main factor influencing the nitrite

Table 3. Residual nitrite levels (mg per kg sausage) in the samples (3 replicates) during the 30 days of storage at 4ºC.[a]

Samples[b]	Storage period (Days)				
	2	9	16	23	30
S1	12.9 ± 1.4^{d1}	11.8 ± 1.1^{bc1}	8.7 ± 0.9^{c2}	6.7 ± 1.1^{d23}	5.0 ± 0.4^{dc3}
S2	12.1 ± 1.1^{d1}	11.4 ± 1.2^{d1}	8.1 ± 1.3^{c2}	6.5 ± 0.2^{d23}	4.9 ± 0.6^{dc3}
S3	13.39 ± 0.9^{d1}	11.3 ± 1.7^{d1}	7.1 ± 0.7^{c2}	6.2 ± 0.3^{d2}	4.7 ± 0.1^{d2}
S4	26.4 ± 2.9^{c1}	19.9 ± 0.5^{b2}	13.6 ± 1.3^{c3}	10.1 ± 0.4^{c34}	8.2 ± 0.2^{c4}
S5	25.2 ± 2.7^{c1}	18.9 ± 0.4^{b2}	13.2 ± 1.4^{c3}	10.0 ± 0.7^{c34}	7.9 ± 0.3^{dc4}
S6	22.3 ± 3.3^{c1}	17.6 ± 0.9^{bc1}	12.4 ± 2.0^{c2}	9.5 ± 0.9^{c23}	7.4 ± 0.5^{dc3}
S7	46.0 ± 4.5^{b1}	32.9 ± 2.1^{a2}	25.8 ± 3.5^{b3}	20.5 ± 1.0^{b34}	16.2 ± 0.4^{b4}
S8	43.3 ± 1.4^{b1}	32.6 ± 2.7^{a2}	25.9 ± 4.4^{b3}	20.0 ± 0.9^{b34}	15.8 ± 0.5^{b4}
S9	42.9 ± 1.4^{b1}	31.6 ± 1.0^{a2}	24.7 ± 2.3^{b3}	19.6 ± 0.6^{b4}	15.1 ± 0.7^{b5}
Control	68.9 ± 1.4^{a1}	54.1 ± 7.1^{a12}	39.0 ± 10.2^{a23}	31.3 ± 1.1^{a34}	23.4 ± 4.1^{a4}
Blank	0.0	0.0	0.0	0.0	0.0

[a] In each column, means identified with different letters are significantly different (P< 0.05). In each row, means identified with different numbers are significantly different (P<0.05). [b] Sample symbols are defined in Table 1.

concentration. Nitrites in the meat form nitric oxide, which reacts with heme compounds in the meat and form nitrosomyoglobin, the pigment responsible for the pink color of cured meats (Gotterup *et al.*, 2008). The nitrite content of the control sample was found at 69 mg kg^{-1} at the beginning of storage period (day 2) while it was reduced to 39 mg kg^{-1} after 16 days of storage. At the end of the storage period (30 days), the nitrite content was reduced to 23 mg kg^{-1}. These results confirmed the findings reported by Cassens (1997) that nitrite level declines during the storage.

Changes in the Microbial Aspects of Cooked Beef Sausages

Changes in the total viable counts of the cooked beef sausages during the 30 days of storage at 4ºC are shown in Table 4. The growth of microorganisms in the blank was more pronounced (P< 0.05) than in the samples with added barberry extract and nitrite at all times. At the end of the storage period, samples S3 and S6 containing the highest levels of barberry extract showed the lowest TVC. Based on the results of the current study, combination of low moisture content, a pH value of around 6.0, and the cooking as well as the storage at 4°C seems to be sufficient to produce a microbiologically stable product for at least 30 days of storage. In most samples, TVC (aerobic) of the bacteria at the end of the storage period was lower than what is considered for a degraded product (< 5 log cfu g^{-1}) (ISIRI, 2010). No indication of *C. perfrigenes* was found in the samples used in the current study. As is the case for lipid oxidation, the antimicrobial activity of barberry extracts might be attributed to the bioactive compounds, especially phenolic compounds and alkaloids that are present in them (Mollaei *et al*., 2010). Also, the effectiveness of antioxidant compounds depends on their corresponding pH values (Li *et al*., 2009). pH values of the cooked sausages from the current study increased gradually during the storage at 4°C (Table 5). This is probably due to the microbial production of basic compounds such as ammonia (Nychas *et al*., 1998) in the product. The values of pH for the control and those for the samples with different concentrations of barberry extracts were

Table 4. Total viable counts (log_{10}cfu/g) of the cooked beef sausages (3 replicates) during the 30 days of storage at 4ºC.[a]

Samples[b]	Storage period (Days)				
	2	9	16	23	30
S1	2.4 ± 0.3^{ab1}	3.42 ± 0.29^{ab2}	3.93 ± 0.37^{ab23}	4.69 ± 0.45^{ab3}	5.83 ± 0.23^{a4}
S2	2.4 ± 0.5^{ab1}	3.12 ± 0.50^{abc12}	3.79 ± 0.36^{abc2}	4.36 ± 0.39^{abc23}	5.34 ± 0.54^{ab3}
S3	2.2 ± 0.3^{b1}	2.46 ± 0.37^{cb1}	2.95 ± 0.27^{c12}	3.45 ± 0.43^{c23}	4.16 ± 0.28^{d3}
S4	2.4 ± 0.4^{ab1}	3.02 ± 0.66^{abc12}	3.53 ± 0.30^{abc12}	4.12 ± 0.51^{abc23}	5.10 ± 0.40^{ab3}
S5	2.6 ± 0.5^{ab1}	3.10 ± 21^{abc12}	3.48 ± 0.23^{bc12}	3.93 ± 0.47^{abc2}	4.89 ± 0.51^{dcb3}
S6	2.2 ± 0.2^{b1}	2.24 ± 0.13^{a1}	2.92 ± 0.11^{a2}	3.32 ± 0.38^{c2}	4.08 ± 0.24^{d3}
S7	2.4 ± 0.3^{ab1}	3.01 ± 0.26^{abc12}	3.32 ± 0.48^{abc12}	4.13 ± 0.44^{abc2}	5.31 ± 0.25^{ab3}
S8	2.3 ± 0.6^{ab1}	2.98 ± 0.28^{abc1}	3.26 ± 0.41^{bc12}	4.08 ± 0.50^{abc2}	5.19 ± 0.29^{ab3}
S9	2.6 ± 0.4^{ab1}	3.03 ± 0.65^{abc12}	3.29 ± 0.57^{abc12}	4.21 ± 0.73^{abc2}	4.95 ± 0.26^{bc3}
Control	2.1 ± 0.2^{ab1}	2.49 ± 0.34^{bc1}	3.16 ± 0.26^{bc2}	3.75 ± 0.48^{bc2}	4.64 ± 0.29^{bc3}
Blank	3.2 ± 0.3^{a1}	3.74 ± 0.18^{a12}	4.18 ± 0.55^{a2}	5.17 ± 0.58^{a3}	6.76 ± 0.41^{a4}

[a] In each column, means identified with different letters are significantly different (P< 0.05). In each row, means identified with different numbers are significantly different (P<0.05). [b] Sample symbols are defined in Table 1.

Table 5. pH values for the cooked beef sausages (3 replicates) during the 30 days of storage at 4ºC.[a]

Samples[b]	Storage period (days)				
	2	9	16	23	30
S1	6.18 ± 0.03^{ab3}	6.29 ± 0.01^{a2}	6.37 ± 0.01^{c2}	6.34 ± 0.10^{dcb2}	6.55 ± 0.04^{b1}
S2	6.17 ± 0.01^{ab3}	6.32 ± 0.02^{a2}	6.35 ± 0.01^{ab2}	6.34 ± 0.00^{dcb2}	6.50 ± 0.08^{cb1}
S3	6.13 ± 0.02^{b3}	6.31 ± 0.02^{a2}	6.32 ± 0.01^{a2}	6.29 ± 0.06^{d2}	6.43 ± 0.04^{a1}
S4	6.19 ± 0.03^{ab3}	6.35 ± 0.05^{a2}	6.34 ± 0.02^{ab2}	6.36 ± 0.04^{bc2}	6.47 ± 0.05^{bc1}
S5	6.20 ± 0.06^{a3}	6.32 ± 0.02^{a12}	6.36 ± 0.03^{b2}	6.31 ± 0.01^{dcb12}	6.49 ± 0.04^{bc1}
S6	6.18 ± 0.03^{ab3}	6.35 ± 0.05^{a2}	6.31 ± 0.01^{ab2}	6.32 ± 0.07^{dcb2}	6.44 ± 0.10^{bc1}
S7	6.23 ± 0.02^{a3}	6.36 ± 0.08^{a2}	6.34 ± 0.04^{ab2}	6.35 ± 0.06^{bc2}	6.51 ± 0.04^{bc1}
S8	6.21 ± 0.03^{a2}	6.29 ± 0.07^{a2}	6.33 ± 0.02^{ab2}	6.33 ± 0.09^{dcb2}	6.51 ± 0.07^{bc1}
S9	6.16 ± 0.01^{ab4}	6.31 ± 0.02^{a3}	6.30 ± 0.06^{ab3}	6.38 ± 0.03^{bc2}	6.52 ± 0.10^{bc1}
Control	6.18 ± 0.03^{ab3}	6.33 ± 0.03^{a2}	6.37 ± 0.02^{ab2}	6.41 ± 0.04^{b2}	6.47 ± 0.00^{bc1}
Blank	6.19 ± 0.03^{ab5}	6.35 ± 0.02^{a4}	6.44 ± 0.02^{a3}	6.56 ± 0.03^{a2}	6.71 ± 0.01^{a1}

[a] In each column, means identified with different letters are significantly different (P< 0.05). In each row, means identified with different numbers are significantly different (P<0.05). [b] Sample symbols are defined in Table 1.

similar in the current study.

Treatments Effects on Antioxidant Properties of the Sausages

Figure 1 shows the effects of barberry extract and added sodium nitrite on the antioxidant properties (DPPH inhibition ratio) of the cooked sausage samples stored at 4°C for 30 days. On the second day of storage, samples with different levels of barberry extract showed higher antioxidant activities than that of the blank. Increasing the extract level in the product (from 30 to 90 mg kg^{-1}) also resulted in an increase in the antioxidant activities of the sausage samples (Figure 1). However, they declined in most samples during the storage. Samples with the highest amount of nitrite (90 mg kg^{-1}) indicated less antioxidant activities than those with 30 and 60 mg kg^{-1} nitrite. This could be explained by possible interaction between the nitrite and the phenolic compounds present in the barberry extract (Coutinho de Oliveira *et al*., 2012). Similar pattern was found in the changes in the TBARS values of the samples studied here during the 30 days of storage (Figure 2). Higher TBARS value for the blank sample indicated that lipid oxidation in this sample was at higher level than those of the other

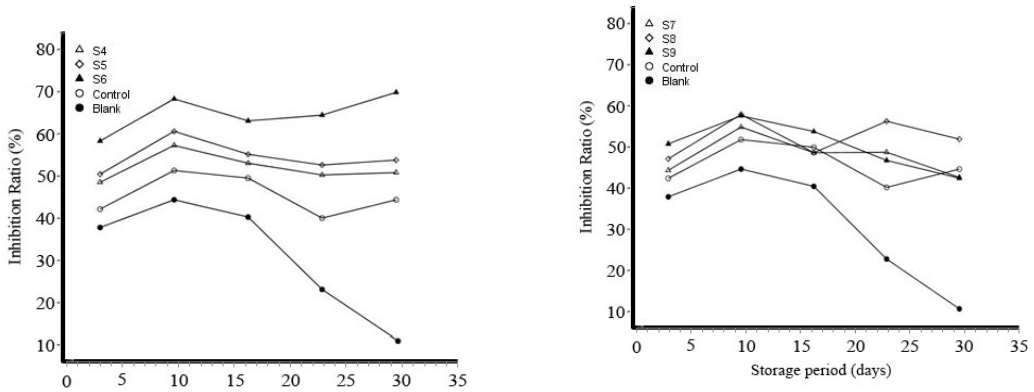

Figure 1. Effects of combined use of barberry extract and sodium nitrite on the antioxidant properties (DPPH inhibition) of the cooked beef sausages stored at 4°C for 30 days (sample identities are given in Table 1).

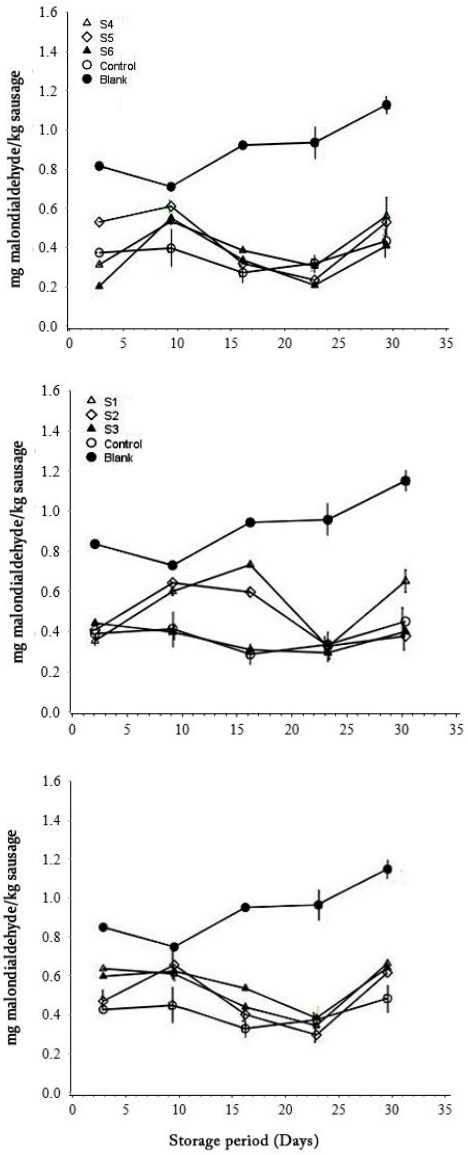

Figure 2. Effects of combined use of barberry extract and sodium nitrite on the *TBARS* values (mg malonaldehyde kg^{-1}) of the cooked beef sausage samples stored at 4°C for 30 days (sample identities are given in Table 1).

samples. Slight differences were found in the TBARS values of the samples with different levels of barberry extract and added nitrite. These results demonstrate the potential antioxidant effect of black barberry extract due to the presence of natural antioxidants (Gulsoy and Ozkan, 2011). According to Lücke (2000), nitrite concentration required for a clear antioxidant effect in different meat products can vary within 20-50 mg kg^{-1}. The maximum allowed level of TBARS value for meat products is about 1 mg malondialdehyde/kg sample (Ockerman, 1976). In the current study, all the samples using nitrite and barberry extract indicated TBARS values below 0.71 mg malondialdehyde kg^{-1} sample and, therefore, they are acceptable as food products. The TBARS values for the samples indicated a decreasing trend from day 9 to day 23 due to a possible malondialdehyde bio-decomposition (Smith and Alford, 1968; Moerck and Ball, 1974), malondialdehyde reactions with proteins and sugars or further oxidation of malondialdehyde to other products such as alcohols and acids (Fernández, *et al*.,1997). Melton (1983) stated that despite the fact that malondialdehyde is a secondary oxidation product; there is no reason for its increase toward the end of storage period. Typically, TBARS values decreased with increasing the concentration of barberry extract in the current study. Although treatment with sodium nitrite and barberry extract resulted in significant inhibition of lipid oxidation ($P<0.05$), the antioxidant effect was only synergistic with the combined effect of 90 mg kg^{-1} extract and 30 mg kg^{-1} nitrite. This combination showed lower ($P< 0.05$) TBARS values than those of other combinations after 30 days of storage. Use of 90 mg kg^{-1} nitrite and barberry extract (combined) helped prevent the lipid oxidation in the current study. However, an antagonistic effect was observed in the samples with 90 mg kg^{-1} barberry extract, which suggests a possible interaction between nitrite and chemical compounds present in the barberry extract. Also, Coutinho de Oliveira *et al* (2012) observed that the highest levels of *Satureja Montana* essential oil and nitrite had antagonistic effect on mortadella.

Treatments Effect on the Color Profile of the Sausages

After microbiological analysis and chemical composition measurements, S3, S6, S9, control and blank samples were chosen for determining the color stability during 30 days of storage at 4ºC. These changes as well as those of the control and the blank samples are shown in Table 6. On the second day of storage (the day after production), samples S3, S6 and S9 had smaller *L** values than the control. This may have been caused by the addition of barberry extract, which presented a dark red color. No significant changes in the Lightness values (L*) were observed during the 30 days of storage for S3, S6, S9 and the control ($P<0.05$). However, the *L** value of the blank decreased during the storage, which may be attributed to the moisture loss. Considering the redness (a* value, the second color attribute investigated in this study), no significant ($P< 0.05$) differences were observed among S3, S6 and the control samples on day 2 of storage. S9, with 90 mg kg^{-1} sodium nitrite, indicated the lowest *a** value compared to S3 and S6 due to higher TBARS values at the end of the storage (0.59, 0.39 and 0.42 mg malondialdehyde kg^{-1}, for S9, S3 and S6, respectively, Figure 2). S3 did not indicate any changes in the redness during the 30 days of storage period, but, S9, the control, and blank samples showed significant reductions during this period. Fernandez-Gines *et al*. (2003) and Terns *et al.* (2011) also observed a similar behavior in the *a** values of sausage throughout the storage. According to Lynch and Faustman (2000), the decline in the intensity of the red color during the storage could be explained by the interdependence between the lipid oxidation and color oxidation in the meats. Pigment oxidation may catalyze lipid oxidation and the free radicals produced during the oxidation may oxidize iron or denature the myoglobin molecules impacting negatively the color of

Table 6. Color attributes (L*, a* and b*) of the selected sausage samples (3 replicates) from this study during the 30 days of storage at 4ºC.[a]

Parameters	Samples[b]	Storage period (Days)				
		2	9	16	23	30
*L**	S3	65.8 ± 1.1^{c1}	64.2 ± 0.9^{b1}	66.0 ± 1.4^{b1}	64.8 ± 1.2^{b1}	64.1 ± 0.9^{b1}
	S6	64.5 ± 0.6^{c1}	64.1 ± 0.7^{b1}	64.7 ± 1.2^{b1}	64.8 ± 0.6^{b1}	63.8 ± 1.5^{b1}
	S9	64.8 ± 0.4^{c1}	64.7 ± 0.3^{b1}	65.4 ± 0.6^{b1}	64.9 ± 1.8^{b1}	63.9 ± 0.4^{b1}
	Control	71.7 ± 0.2^{a1}	70.4 ± 1.1^{a1}	71.9 ± 0.3^{a1}	69.8 ± 1.4^{a1}	69.1 ± 2.1^{a1}
	Blank	57.1 ± 0.4^{c1}	54.0 ± 0.84^{c2}	52.1 ± 0.2^{c3}	49.7 ± 0.7^{c2}	47.9 ± 1.1^{c2}
*a**	S3	9.3 ± 0.9^{a1}	9.9 ± 0.2^{a1}	9.4 ± 0.1^{b1}	9.4 ± 0.0^{ab1}	9.9 ± 0.2^{a1}
	S6	9.4 ± 0.1^{a1}	9.3 ± 0.4^{a1}	9.8 ± 0.3^{a12}	9.7 ± 0.2^{a12}	10.1 ± 0.2^{a2}
	S9	9.0 ± 0.1^{a4}	8.6 ± 0.1^{b3}	8.4 ± 0.1^{c2}	8.5 ± 0.1^{c23}	7.9 ± 0.00^{c1}
	Control	9.1 ± 0.2^{a12}	9.6 ± 0.3^{a2}	9.5 ± 0.1^{ab2}	9.1 ± 0.2^{b12}	8.7 ± 0.3^{b1}
	Blank	4.3 ± 0.1^{b3}	5.2 ± 0.1^{b4}	4.2 ± 0.1^{d3}	3.5 ± 0.2^{d2}	2.1 ± 0.4^{d1}
*b**	S3	10.1 ± 0.5^{a1}	9.9 ± 0.1^{b12}	9.8 ± 0.3^{b12}	9.8 ± 0.1^{b12}	9.4 ± 0.3^{bc2}
	S6	10.1 ± 0.1^{a1}	9.8 ± 0.1^{b1}	10.0 ± 0.1^{b1}	9.6 ± 0.3^{bc1}	9.6 ± 0.5^{b1}
	S9	10.7 ± 0.4^{ab1}	9.6 ± 0.9^{b12}	9.9 ± 0.1^{b12}	9.3 ± 0.5^{c2}	8.7 ± 0.1^{c3}
	Control	10.9 ± 0.1^{b1}	10.1 ± 0.1^{b2}	9.1 ± 0.1^{c3}	9.6 ± 0.0^{bc23}	10.1 ± 0.4^{ab2}
	Blank	11.8 ± 0.1^{a12}	11.5 ± 0.1^{a2}	11.9 ± 0.2^{a1}	11.2 ± 0.1^{a3}	10.7 ± 0.2^{a4}

[a] In each column, means identified with different letters are significantly different ($P< 0.05$). In each row, means identified with different numbers are significantly different ($P<0.05$). [b] Sample symbols are defined in Table 1.

Table 7. Sensory evaluation of the selected sausage samples (3 replicates).[a]

Sample[b]	Color	Flavor		Overall acceptability
		Odor	Taste	
S3	6.5±0.6^{ab}	8.2±0.6^{a}	6.4±0.7^{a}	7.3±1^{a}
S6	6.6±0.6^{ab}	8.2±0.6^{a}	6.3±0.8^{ab}	7.2±0.7^{a}
S9	6.2±0.8^{b}	8.3±0.7^{a}	5.9±0.8^{b}	6.5±1^{b}
Control	6.6±0.7^{a}	8.1±0.9^{a}	5.8±0.8^{b}	7.1±1.3^{ab}
Blank	3.8±0.7^{c}	7.5±0.2^{b}	4.3±0.9^{c}	5.3±1^{c}

[a] In each column, means identified with different letters are significantly different (P< 0.05).
[b] Sample symbols are defined in Table 1.

the products (Lynch and Faustman, 2000). Slight increase in the *a** value was observed for S6 during the storage, indicating a color change from red to brown possibly due to the formation of metmyoglobin (Hunt *et al*.,1999). Under a reducing condition, a dark red color is observed due to the formation of nitric oxide myoglobin (Schmidt, 1986). Yellowness (b* value) is another color attribute considered in the current study. On day 2, S3 and S6 indicated slightly lower yellowness when compared to the control sample. Except for S6, all samples of this study lost their yellowness during the 30 days of storage (Table 6). In general, the results of the instrumental yellow color (b*) measurements followed the TBARS values. Lauritzen and Martinsen (1999) reported that the yellowness was correlated with lipid oxidation in cod fillets during the salting process, with increases in lipid oxidation raising yellow pigment formation.

Yu *et al*. (28) also reported that lipid oxidation correlated with an increase in *b** values in cooked turkey products during refrigerated storage. Yellow pigment in the meat product is produced by the non-enzymatic browning reactions occurring between lipid oxidation products and amino groups of the proteins.

Treatments Effect on the Sensory Attributes of the Sausages

The results for the sensory evaluation of the sausage samples from the current study are shown in Table 7. The taste score given for S3 sample was lower than that of the control. No significant differences were found in flavor of S6, S9, and the control samples (Table 7). Scores given for the odor of the samples S3, S6, S9, and the control were not different. Overall score for the flavor of blank sample was lower than those of the other samples. Considering the overall acceptability of the samples, S3, S6, and the control received higher scores than S9 and blank.

CONCLUSIONS

This study showed that high level of barberry extract (90 mg kg^{-1}) with a low level of nitrite (30 or 60 mg kg^{-1}) can be used effectively to extend the shelf-life of cooked sausages if stored under refrigerated conditions (4ºC). Barberry extract resulted in an increase in the antioxidant activities of the samples suggesting that partial replacement of nitrite with this extract can result in a healthier product, potentially reducing the risk of cancer due to the reduced nitrosamine formation in the sausage products. Samples with 90 mg kg^{-1} barberry extract and the lowest level of nitrite (30 and 60 mg kg^{-1}) were granted the highest score in the sensory evaluation of the samples.

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تأثیر مشترک عصاره زرشک سیاه و نیتریت بر روی کیفیت و زمان ماندگاری سوسیس پخته در طی نگهداری در دمای یخچال

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چکیده

اثر مشترک ترکیب عصاره زرشک سیاه (*Berberis crataegina*) و نیتریت سدیم روی کیفیت و زمان ماندگاری سوسیس های پخته شده مورد بررسی قرار گرفت. غلظت های متفاوتی از این عصاره در ترکیب با نیتریت به فرمولاسیون سوسیس افزوده شد. شمارش کل میکروبی، pH، آنالیز تقریبی، سطح نیتریت باقیمانده، اکسیداسیون لیپید، رنگ و داده های حسی در برابر نمونه شاهد و کنترل در طی نگهداری در مدت ۳۰ روز در دمای $4\ ^{\circ}C$ مورد مطالعه قرار گرفت. کاهش تدریجی سطح نیتریت در طی مدت زمان نگهداری در همه نمونه های مورد مطالعه مشاهده شد. نمونه هایی که عصاره زرشک سیاه در آنها به کار رفته بود در مقایسه با نمونه سوسیس شاهد رنگ قرمز مشابه با میزان روشنی کمتری داشتند. ارزیابی حسی نمونه ها نتایج مشابه با نمونه های کنترل را نشان داد. برتری بالقوه ای در جایگزین کردن این عصاره با بخشی از نیتریت سدیم در محصولات گوشتی فرآوری شده مشاهده شد.