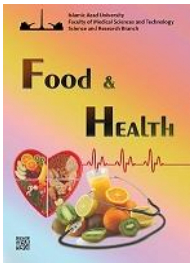

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Quality assessment of unfermented sausage by partial replacement of sodium nitrite with ethanolic extracts of *Rosmarinus officinalis* and *Berberis vulgaris*

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ABSTRACT

In this research, nitrite was partially replaced with different concentrations of *Berberis vulgaris* and *Rosmarinus officinalis* (0, 30, 60, 90 mg/kg) extracts in the unfermented sausage. Samples were kept at refrigerator temperature and physicochemical and organoleptic evaluations of treatments were performed. The findings showed an increase in moisture and ash content and a decrease in fat and protein content, and IC_{50} by increasing the storage time. The highest level of antioxidant activity was reported for treatment with 90 mg/kg *R. officinalis* extract. As for sensory properties, 60 mg/kg treatment showed the highest sensory score after the control sample; however, 30 mg/kg treatment had the highest score in terms of consistency after the control sample. It can be concluded that 60 mg/kg *R. officinalis* extract has a better potential to improve the qualitative characteristics of unfermented sausage and it can be a proper substitute for decreasing the nitrite in sausage formulations.

1. Introduction

The consumption of animal products including meat and meat products has been increased globally with an increase in household income (1), although others prefer meatless diets for different reasons (2). One of these products is sausage with an increasing consumption around the world owing to its lower cost and more favorable taste than meat as well as easy and even quick preparation (3). Sausage is a good source of protein with high quality that contains all amino acids at appropriate levels and is required for the growth, maintenance, and regeneration of body tissues. Furthermore, it supplies a significant amount of the body's requirements for vitamins and nutrients (4). This product, as a good source of nutrients, is an appropriate environment for the growth of spoilage and pathogenic microorganisms (5, 6). In addition to the preservative action, particularly against *Clostridium botulinum*, the curing process imparts several other distinctive properties that are common to all cured meat products which are attributable to the sodium nitrite present in the curing mixture (7). Other properties include a contribution to the formation of a unique color, texture, and flavor to cured meat products and protection of meat lipids from oxidation (8). According to Sindelar and Milkowski (9), the limit of sodium nitrite to obtain the required cured meat flavor and oxidative stability is above 50 mg/kg. The influence of nitrite on the flavor of meats is well described, but the chemical movements are still unclear. Because of the concerns about chemical preservatives and their possible harmful effects, there has been a growing interest in the use of natural preservatives (10, 11). Plant medicines have been taken into account in different forms such as powder, extract, and essence, owing to having effective compounds like polyphenols, tannins, flavonoids, and phenolic acids, as a major source of antioxidant and antimicrobial compounds with various properties, especially anti-mutagenic and anti-cancer ones (12). They have also been found to be effective in delaying the physicochemical, oxidative, and microbial changes and increasing the shelf life of food products (13, 14). *Berberis vulgaris* is a member of the *Berberidaceae* family. It is well-known in Iran and Turkey and

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is also known as barberry or dehydrated barberry in Iran. *B. vulgaris* possesses high antimicrobial and antioxidant properties (15). *Rosmarinus officinalis* belongs to the mint family and is a perennial shrub grown all over Iran (16). Among the plant medicines, *R. officinalis* has high antimicrobial properties, and various reports have documented this antimicrobial activity against both gram-positive and gram-negative bacteria (17, 18). The extract of the leaves of this plant contains phenolic compounds such as rosmarinic acid, carnosic acid, and carnosol (12), and its different biological properties such as antioxidant, disinfectant, anti-spasm, and anti-inflammatory effects have been studied and confirmed (19). Considering the known risks of nitrite and sodium nitrite for the health of consumers during long-term periods, it is necessary to replace these synthetic compounds with natural plant sources with antioxidant and antimicrobial properties in the formulation of meat products. Hence, this study was an attempt to replace part of sodium nitrite in the formulation of unfermented sausage with *B. vulgaris* and *R. officinalis* extracts and to evaluate their potential in boosting the qualitative characteristics and acceptability of the final products for the consumers.

2. Materials and methods

2.1. Extraction of Berberis vulgaris

B. vulgaris fruit was purchased from a local market in Karaj, Iran, dried at ambient temperature over two weeks, and transferred to a food laboratory afterward. The dried samples were packaged in multilayer packages to prevent the penetration of humidity and kept at -20°C until the experiments were done. For extraction, first, the dried samples were powdered by an electric grinder and sieved by a sieve no 20. Next, an adequate amount of etherdopetrol was added to the obtained powder so that it covered all the powder surfaces. The samples were stirred in a closed balloon for 12 h. Then, the solution was isolated from the powder and dried under a hood. Next, the powder was mixed with ethanol with a 1:10 ratio and stirred for 24 h at room temperature in dark. The obtained extract was filtered by Whatman filter paper grade 1. The filtered extract was dried in a rotary vacuum evaporator at 40°C and its solvent was isolated (15).

2.2. Extract of Rosmarinus officinalis

The leaves of *R. officinalis* were purchased from an authentic herb store in Kashan, Iran, were first confirmed by the researcher of the medicinal plant research department of the National Research Institute of Forests and Rangelands (Karaj, Alborz, Iran). The leaves were delivered to the Science and Technology Park laboratory of Tehran University (Karaj, Alborz, Iran), and was dried in a cool and dark environment, and powdered by an electric grinder. The powder was mixed with 60% ethanol and extracted by a Soxhlet extractor for 10 min. Then, the ethanolic phase was isolated from the extract by a vacuum concentrator and poured into a decanter funnel to remove the fat phase and pigments. Hexane was added to the decanter half the extract volume and stirred well. The hexane phase was isolated and the obtained extract was dried in the vacuum concentrator (20). After preparing the treatments and control samples, the following experiments were conducted on days 1, 10, 20, and 30.

2.3. Preparation of treatments

The studied treatments are presented in Table 1. The overall formulation included 60% beef and other ingredients, including 18.51% water and ice, 14% soybean oil, 1.5% salt, 2.8% starch, 1.8% soybean isolate, 0.4% sodium phosphate, 0.05% ascorbic acid, and 0.9% different spices. All the substances were mixed in a cutter, and the obtained paste was mixed with nitrite, the studied extracts, and half of the remaining water and ice in separate batches in the cutter. All batches were separately packaged in polyamide packages and cooked at 75°C for 1 h. Then, they were cooled under cold water and transferred to and kept in a refrigerator for 30 days (21).

Table 1. Characteristics of treatments.

Treatment	Nitrite (mg/kg)	Rosmarinus *officinalis* (mg/kg)	Berberis *vulgaris* (mg/kg)
1	90	·	30
2	60	·	60
3	30	·	90
4	90	30	·
5	60	60	·
6	30	90	·
Control	120	0	0

2.4. Physiochemical tests

2.4.1.Determination of pH value

The pH test was performed according to standard method NO.1028, Institute of Standards and Industrial Research of Iran (22). 5 g of homogenized sample with 20 ml of distilled water was mixed well. The pH of the homogenate was measured using a pH electrode (Mettler-Toledo, Inlab Semi-Micro Electrode, Greifensee, Switzerland) attached to a pH meter (Mettler-Toledo, S40 SevenMulti™, Greifensee, Switzerland).

2.4.2.Determination of Moisture Content

The samples were discharged between 20 and 25 grams inside the plate and placed in an autoclave for 30 min at a temperature of 103 ± 2°C. Then, it was placed inside the desiccator to cool. Then, take 5 to 8 g of the sample and place it in an autoclave at a temperature of 103 ± 2°C for 2 h .Then the plate was inserted into the desiccator to cool. Ultimately weighed (23).

2.4.3.Determination of Protein Content

At first, about 2 grams of sample were prepared. 20 ml of

concentrated sulfuric acid solution and 8 g of a catalyst mixture (potassium sulfate 96%, copper sulfate 3.5%, and selenium oxide 0.5%) were added and the balloon was attached to Kjeldahl digestion equipment and heated. The balloon was cooled and then washed with 166 ml of distilled water repeatedly and was poured into the container through a funnel of distillation balloons. The balloon was heated and continue to distill, until the end of the condenser is in a boric acid solution, to collect all the ammonia in the receptacle . About 200-250 ml of distilled solution was collected and titrated with sulfuric acid solution 0.1N (24).

2.4.4.Determination of fat content

5 g of the sample was weighed in a 250 mL Erlenmeyer flask and 50 ml of chloride was added. The extraction tube was attached to the Erlenmeyer and placed on a flame for 4 h. After extraction, the Erlenmeyer contents were distilled. After complete solvent evaporation, fat content was calculated using the Soxhlet method based on the ISIRI standard (No. 760) with SAL of ≤1 (g/percent) (25).

2.4.5.Determination of ash content

At first, 1.5-2 g of the sample was uniformly spread inside the crucible and heated from 20°C in an electric furnace to 550 ±25°C. It was then cooled in the desiccator at ambient temperature and then weighed by laboratory scale (26).

2.4.6.DPPH radical scavenging activity

DPPH is a lipophilic radical showing the peak absorbance at 517nm. Hydroxyl group of antioxidant compounds reduced DPPH by donating hydrogen to free radical DPPH demonstrated by the color change of reaction solution from dark purple to bright yellow. Therefore, absorbance at 517 nm decreased (27). In this study, 40 µl of *Berberis vulgaris and Rosmarinus officinalis* extract were transferred to a test tube to which 1ml of 0/2 mmol DPPH was added. Absorbance declined at 517nm after 30min. The absorbance of DPPH without extract was considered as a control. Radical scavenging was calculated by the following formula:

$$\text{DPPH scavenging (\%)} = ((A_0-A_1) / A_0) \times 100 \quad \text{(Eq. 1)}$$

where, A_0 is the absorbance without extract (blank) and A_1 is the absorbance of antioxidative extract.

2.5. Sensory evaluation

The sausage samples were assessed by 12 trained panelists. The assessment forms were prepared based on the 5-point hedonic scale, including 1: unacceptable, 2: relatively good, 3: good, 4: very good, and 5: excellent. The samples were wrapped with foil and placed in the oven for 8 min. They were then removed from the oven and cut into 2 cm-thick sections after reaching the ambient temperature. The sensory parameters included the color, flavor, consistency, and overall acceptance of the samples (28).

2.6. Statistical analysis

A one-way analysis of variance (ANOVA) with two factors (treatments and storage period) was applied for each parameter using the SPSS program (ver. 26). Means and standard errors were calculated and a probability level of $p<0.05$ was used in testing the statistical significance of all experimental data. Excel software (2010) was used for drawing the tables and figures.

3. Results and discussion

3.1. pH value

According to Table 2, with increasing storage time, except for the control sample and treatment containing 90 ppm rosemary extract, the trend of pH value changes was decreasing. The latter treatment showed the highest mean pH value over a month. On the last day, there was no significant difference between the pH values of the treatments containing barberry and rosemary extract ($p>0.05$).

Table 2. Mean comparison of pH value of treatments during storage.

Treatments	Time (Day)			
	1	10	20	30
1	6.14±0.003^{c}	6.12±0.002^{bc}	6.10±0.003^{d}	6.02±0.012^{b}
2	6.16±0.003^{a*}	6.12±0.002^{bc}	6.11±0.003^{cd}	6.00±0.012^{b}
3	6.15±0.003^{bc}	6.12±0.002^{bc}	6.12±0.003^{c}	6.02±0.012^{b}
4	6.16±0.003^{ab}	6.14±0.002^{a}	6.10±0.003^{d}	6.10±0.012^{a}
5	6.12±0.003^{e}	6.11±0.002^{c}	6.12±0.003^{bc}	6.10±0.012^{a}
6	6.12±0.003^{de}	6.11±0.002^{c}	6.15±0.003^{a}	6.13±0.012^{a}
Control	6.13±0.003^{cd}	6.13±0.002^{ab}	6.14±0.003^{b}	6.13±0.012^{a}

*Different letters in each column indicate a significant difference ($p<0.05$).

One of the most important reasons for lowering the pH value, is the predominance of lactic acid bacteria, the accumulation of organic acids as well as the accumulation of lipid oxidation products (29). The decrease in pH value in all treatments compared to the control sample was due to the addition of extracts that had acidic nature. This result is consistent with the results of studies by some researchers such as Ragab et al. (30), Sucu and Turp (31), Kim et al. (32), and

Emiroglu et al. (33). The most important reasons for the pH reduction of sausage samples are the dominance of lactic acid bacteria and the accumulation of organic acids and products derived from lipid oxidation. This result is in line with the findings of some researchers like Ragab et al. (30). The addition of herbal extracts decreased the pH value (34). The reducing pH value might be due to the accumulation of metabolites by bacterial activity in meat, which results from protein and amino acid degradation. Contrary to these results, Mohamed and Mansour (35) reported that no significant differences were observed in the pH values of beef patties after incorporating natural herbal extracts (29). But the increase in pH value over time for the storage time of meat products is often due to the production of substances such as ammonia derived from the activity of microorganisms, protein denaturation, and accumulation of raw materials (36).

3.2. Moisture content

According to Table 3, With increasing shelf life from day 1 to 30, the moisture content of treatments evaluated decreased . The highest mean during the storage time belonged to the control sample and the lowest mean related to treatment containing 60 ppm rosemary extract .In all days studied, this treatment had a significant difference with the control sample ($p<0.05$).

Table 3. Mean comparison of moisture content (%) of treatments during storage.

Treatments	Time (Day)			
	1	10	20	30
1	65.07a±0.16	63.05c±0.3	62.10b±0.2	59.19b±0.2
2	64.00b±0.16	63.89b±0.3	62.06b±0.2	60.00a±0.2
3	65.08a±0.16	63.85b±0.3	62.03b±0.2	60.04a±0.2
4	63.00c±0.16	62.14d±0.3	62.00b±0.2	60.11a±0.2
5	64.04a±0.16	57.67ab±0.1	60.09c±0.2	58.14c±0.2
6	65.00a±0.16	62.19d±0.3	60.00c±0.2	57.00d±0.2
Control	65.03a±0.16	65.00a±0.3	64.00a±0.2	60.13a±0.2

*Different letters in each column indicate a significant difference ($p<0.05$).

In the present study, with increasing shelf life during the first month, the moisture content of the evaluated treatments decreased. The lowest level was observed with the treatment containing 60 ppm rosemary extract. As the amount of fat in the sample increases, the water absorption and ultimately the moisture content of the sample will decrease, as the presence of fat will cover the waterproof areas. Therefore, the researchers concluded that by removing fat from meals, the water absorption properties increased.

3.3. Ash content

According to Table 4, the ash content increased over time from day one to thirty. During storage time, the highest and lowest mean were treatment containing 30 ppm of Rosemary extract and control respectively. At the end of the storage period, there was a significant difference between the control sample and all treatments ($p<0.05$). The ash content of food depends on the minerals, proteins, polysaccharides, and salts contents. The ash content of meat was mostly affected by the salt and protein added. Over time, the ash content increased. The ash content of the extract containing samples corresponds to the standard for meat products. In this case, in the study of Pour Mollaei et al. (37), *Origanum vulgare L.* essence (5 %) increased the ash content of surimi samples over time. Viuda-Martos et al. (38) observed that with the addition of 0.02% essential oil of thyme and oregano extracts, the ash content in some samples showed significantly higher rates while protein and fat did not change significantly in any of the samples. Color formation and its stability during cold storage are very

Table 4. Mean comparison of ash content (%) of treatments during storage.

Treatments	Time (Day)			
	1	10	20	30
1	2.21^{a}±0.019	2.26^{a}±0.008	2.23^{c}±0.009	2.28^{c}±0.018
2	2.12^{c}±0.019	2.16^{d}±0.008	2.24^{c}±0.009	2.28^{c}±0.018
3	2.17^{b}±0.019	2.20^{b}±0.008	2.23^{c}±0.009	2.25^{c}±0.018
4	2.16^{b}±0.019	2.21^{b}±0.008	2.31^{a}±0.009	2.44^{a}±0.018
5	2.00^{a}±0.019	2.16^{cd}±0.008	2.32^{a}±0.009	2.48^{a}±0.018
6	2.01^{a}±0.019	2.19^{bc}±0.008	2.29^{ab}±0.009	2.34^{b}±0.018
Control	2.00^{a}±0.019	2.15^{a}±0.008	2.25^{bc}±0.009	2.30^{bc}±0.018

*Different letters in each column indicate a significant difference ($p<0.05$).

important quality attributes of meat products (39). The color of meat products and meat is influenced by metmyoglobin percentage in muscle. Initially, the myoglobin was changed into oxymyoglobin (light pink color), which could result in brighter red meat, and then oxymyoglobin was oxidized into metmyoglobin during storage (40).

3.4. Protein content

According to Table 5, the highest and the lowest mean of protein belonged to the treatments containing 50 and 90 ppm rosemary extract respectively, which had a significant difference in the early and final days with the control sample

($p<0.05$). In general, protein content was higher in treatments containing different levels of rosemary extract compared to blackberry extract. The highest mean belonged to the treatment containing 50 ppm rosemary extract.

Table 5. Mean comparison of protein content (%) of treatments during storage.

Treatments	Time (Day)			
	1	10	20	30
1	$14.55^{a}\pm0.03$	$16.00^{a}\pm0.09$	$17.05^{bc}\pm0.03$	$17.35^{c}\pm0.06$
2	$14.32^{abc}\pm0.03$	$16.37^{a}\pm0.09$	$17.07^{bc}\pm0.03$	$17.15^{d}\pm0.06$
3	$14.25^{bc}\pm0.03$	$16.03^{a}\pm0.09$	$17.00^{c}\pm0.03$	$17.03^{d}\pm0.06$
4	$14.45^{abc}\pm0.03$	$15.76^{a}\pm0.09$	$17.18^{b}\pm0.03$	$17.54^{b}\pm0.06$
5	$14.21^{c}\pm0.03$	$16.20^{a}\pm0.09$	$17.40^{a}\pm0.03$	$17.75^{a}\pm0.06$
6	$14.19^{c}\pm0.03$	$16.07^{a}\pm0.09$	$17.00^{c}\pm0.03$	$17.00^{d}\pm0.06$
Control	$14.47^{bc}\pm0.03$	$16.47^{a}\pm0.09$	$17.00^{c}\pm0.03$	$17.09^{d}\pm0.06$

*Different letters in each column indicate a significant difference ($p<0.05$).

3.5. Fat content

According to Table 6, The fat content decreased during the storage time. The control sample showed the highest fat amount during storage and the difference between treatments was significant during the first ten days ($p<0.05$). During the last ten days, the lowest mean lipid content belonged to the treatment containing 60 ppm rosemary extract. At the end of the storage period, there was no significant difference between the 90 ppm of this extract and control groups ($p>0.05$), but other treatments showed a significant difference ($p>0.05$). The decrease in fat content in the treatments compared to the control sample indicates that oxidation occurred in the meat fat and the fats decomposed.

Table 6. Mean comparison of fat content (%) of treatments during storage.

Treatments	Time (Day)			
	1	10	20	30
1	$20.00^{a}\pm0.03$	$19.14^{b}\pm0.03$	$19.15^{b}\pm0.02$	$18.31^{b}\pm0.05$
2	$20.05^{a}\pm0.03$	$19.00^{b}\pm0.03$	$19.10^{c}\pm0.02$	$18.31^{cd}\pm0.05$
3	$20.01^{a}\pm0.03$	$19.03^{b}\pm0.03$	$19.05^{d}\pm0.02$	$18.13^{c}\pm0.05$
4	$20.00^{a}\pm0.03$	$19.10^{b}\pm0.03$	$19.00^{e}\pm0.02$	$18.016^{cd}\pm0.05$
5	$19.74^{b}\pm0.03$	$19.02^{b}\pm0.03$	$19.01^{de}\pm0.02$	$18.00^{d}\pm0.05$
6	$19.76^{b}\pm0.03$	$19.35^{a}\pm0.03$	$19.00^{e}\pm0.02$	$18.00^{d}\pm0.05$
Control	$20.00^{a}\pm0.03$	$19.47^{a}\pm0.03$	$19.35^{a}\pm0.02$	$18.66^{a}\pm0.05$

*Different letters in each column indicate a significant difference ($p<0.05$).

3.6. DPPH radical scavenging activity

According to Table 7, the radical scavenging activity of the extracts decreased over time. But at the same time, the difference between the control sample and the treatments containing two extracts was significant ($p<0.05$). The highest mean radical scavenging activity during storage time was related to 60 (mg/kg) rosemary extract, which was significantly different from the control and other treatments. In contrast, the control sample had the lowest antioxidant activity or the highest IC_{50}. Studies in red meat have shown that rosemary extract prevents lipid oxidation during storage (41). The manifested antioxidant effect of the tested extracts in sausages was considered significant due to the fact that the sausages obtained from frozen meat and fat tissue stored for a long time easily oxidize, that lipolysis is more intense than in the swine and cattle meat. Free radical scavenging activity is one of the mechanisms involved in inhibiting lipid oxidation and is commonly used to estimate antioxidant activity (42). This result has been reported in researches by Rather et al. (43). In addition, Coutinho de Oliveira et al. (44) observed that the highest levels of *Satureja montana* essential oil and nitrite had an antagonistic effect on mortadella. Antioxidant properties of polyphenolics arise from their high reactivity as hydrogen or electron donors from the ability of polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain braking function) and from their potential to chelate metal ions (Termination of fen-ton reaction). Furthermore, various factors like stereoselectivity of the radicals or the solubility of the tested sample in different testing systems and functional groups present in the bioactive compounds had been reported to affect the capacity of the sample to react and quench different radicals (45). Also, these results agree with that reported by Mc Carthy et al. (46), and Sebranek et al. (47). Karpinska et al. (48) reported that the application of 1.5% of sage alone was more effective on turkey meat dish storage stability than mixture (1 %) of spices (sage, red pepper, black pepper, garlic, and marjoram). Contrary to these results, Simitzis et al. (49) reported that dietary incorporation of oregano essential oil exerted strong antioxidant effects on lipid oxidation in meat during long-term frozen storage. Owing to its excellent protective features exhibited in antioxidant activity tests, the essential oil, and extracts from the herbal parts of *Z. multiflora boiss* can be freely used in the food

industry as a culinary herb (50). In the current study, the sensory scores of treatments were declined during cold storage. In terms of overall acceptance, the treatments with *Z. multiflora* extract had a higher score). The results were similar with Jamwal et al. (51) in chicken meat patties and Nath et al. (52) in chevon patties. Evaporative losses leading to declining in juiciness, the reduction in mean consistency scores during refrigerated storage might be due to the relative reduction in moisture and juiciness of the product that led to the hardening of the product.

Table 7. Mean comparison of DPPH scavenging activity (IC50) (%) of treatments during storage.

Treatments	Time (Day)			
	1	10	20	30
1	$90.33^{a}\pm1.09$	$83.00^{cd}\pm0.95$	$70.66^{c}\pm1.9$	$56.00^{c}\pm1.5$
2	$84.66^{b}\pm1.09$	$85.00^{bcd}\pm0.95$	$80.33^{a}\pm1.9$	$62.33^{b}\pm1.5$
3	$84.66^{b}\pm1.09$	$86.33^{ab}\pm0.95$	$74.33^{b}\pm1.9$	$60.33^{b}\pm1.5$
4	$90.00^{a}\pm1.09$	$88.00^{a}\pm0.95$	$63.33^{d}\pm1.9$	$53.00^{c}\pm1.5$
5	$76.33^{d}\pm1.09$	$74.66^{e}\pm0.95$	$60.00^{e}\pm1.9$	$50.33^{d}\pm1.5$
6	$80.33^{c}\pm1.09$	$82.66^{d}\pm0.95$	$58.00^{f}\pm1.9$	$49.00^{d}\pm1.5$
Control	$86.00^{b}\pm1.09$	$85.66^{abc}\pm0.95$	$79.66^{a}\pm1.9$	$70.33^{a}\pm1.5$

*Different letters in each column indicate a significant difference ($p<0.05$).

3.7. Sensory evaluation

Food acceptability by the consumer is one of the effective aspects of the success of innovations and manipulation of formulations. The highest and lowest scores of colors on the 30 days were related to the treatment containing 60 (mg/kg) blackberry extract and the 30 (mg/kg) rosemary extract. As the storage time increased, the flavor scores of the treatments decreased. At the end of the day, the highest flavor score was related to the treatment containing 90 (mg/kg) rosemary extract, which was significantly different from the control sample ($p<0.05$). As the shelf life increased, the consistency scores of the treatments decreased, which was the highest in the last day of control. Over time, the overall acceptance scores of treatments decreased. The highest overall acceptance score at the end of the day was related to the control sample, which was not significantly different from a treatment containing 60 (mg/kg) of rosemary extract ($p>0.05$). Kant et al. (53) observed that the sausage sample containing peppermint extract did not differ significantly from control samples in taste and flavor. This result can also be interpreted from recent research. Because the addition of different medicinal plant

Table 8. Mean comparison of sensory scores of treatments during storage.

Sensory parameters	Treatments	Time (Day)			
		1	10	20	30
Color scores	1	$5.00^{a}\pm0.00$	$4.82^{a}\pm0.04$	$4.34^{bc}\pm0.24$	$4.00^{a}\pm0.09$
	2	$5.00^{a}\pm0.00$	$4.84^{a}\pm0.04$	$4.44^{ab}\pm0.24$	$4.06^{a}\pm0.09$
	3	$5.00^{a}\pm0.00$	$4.73^{a}\pm0.04$	$4.32^{cd}\pm0.24$	$3.66^{b}\pm0.09$
	4	$5.00^{a}\pm0.00$	$4.29^{c}\pm0.04$	$4.18^{e}\pm0.24$	$3.00^{d}\pm0.09$
	5	$5.00^{a}\pm0.00$	$4.36^{c}\pm0.04$	$4.20^{e}\pm0.24$	$3.19^{c}\pm0.09$
	6	$5.00^{a}\pm0.00$	$4.50^{b}\pm0.04$	$4.23^{de}\pm0.24$	$3.24^{c}\pm0.09$
	Control	$5.00^{a}\pm0.00$	$4.79^{a}\pm0.04$	$4.45^{a}\pm0.24$	$4.05^{a}\pm0.09$
Flavor scores	1	$5.00^{a}\pm0.06$	$4.34^{c}\pm0.05$	$4.22^{b}\pm0.02$	$3.86^{b}\pm0.04$
	2	$5.00^{a}\pm0.06$	$4.60^{b}\pm0.05$	$4.30^{b}\pm0.02$	$4.00^{a}\pm0.04$
	3	$4.52^{b}\pm0.06$	$4.34^{c}\pm0.05$	$4.27^{b}\pm0.02$	$3.79^{bc}\pm0.04$
	4	$4.33^{c}\pm0.06$	$4.11^{de}\pm0.05$	$4.11^{c}\pm0.02$	$3.61^{d}\pm0.04$
	5	$4.85^{a}\pm0.06$	$4.17^{d}\pm0.05$	$4.10^{c}\pm0.02$	$3.73^{bc}\pm0.04$
	6	$4.31^{c}\pm0.06$	$4.08^{e}\pm0.05$	$4.03^{c}\pm0.02$	$3.34^{e}\pm0.04$
	Control	$5.00^{a}\pm0.06$	$4.72^{a}\pm0.05$	$4.41^{a}\pm0.02$	$4.00^{a}\pm0.04$
Consistency scores	1	$5.00^{a}\pm0.00$	$5.00^{a}\pm0.08$	$4.52^{b}\pm0.05$	$4.00^{a}\pm0.05$
	2	$5.00^{a}\pm0.00$	$4.80^{b}\pm0.08$	$4.45^{b}\pm0.05$	$4.00^{a}\pm0.05$
	3	$5.00^{a}\pm0.00$	$439^{c}\pm0.08$	$4.23^{c}\pm0.05$	$3.81^{b}\pm0.05$
	4	$5.00^{a}\pm0.00$	$4.22^{d}\pm0.08$	$4.18^{e}\pm0.24$	$3.00^{d}\pm0.09$
	5	$5.00^{a}\pm0.00$	$4.10^{e}\pm0.08$	$4.20^{e}\pm0.24$	$3.19^{c}\pm0.09$
	6	$5.00^{a}\pm0.00$	$4.10^{e}\pm0.08$	$4.23^{de}\pm0.24$	$3.24^{c}\pm0.09$
	Control	$5.00^{a}\pm0.00$	$5.00^{a}\pm0.08$	$4.45^{a}\pm0.24$	$4.05^{a}\pm0.09$
Overall acceptance	1	$4.25^{c}\pm0.06$	$4.31^{b}\pm0.03$	$4.00^{a}\pm0.07$	$3.55^{b}\pm0.08$
	2	$4.57^{b}\pm0.06$	$4.38^{b}\pm0.03$	$4.00^{a}\pm0.07$	$3.79^{a}\pm0.08$
	3	$4.80^{a}\pm0.06$	$4.58^{a}\pm0.03$	$3.68^{b}\pm0.07$	$3.46^{b}\pm0.08$
	4	$4.36^{c}\pm0.06$	$4.29^{b}\pm0.03$	$3.13^{e}\pm0.07$	$3.10^{d}\pm0.08$
	5	$4.20^{c}\pm0.06$	$4.10^{c}\pm0.03$	$3.44^{c}\pm0.07$	$3.30^{c}\pm0.08$
	6	$4.21^{c}\pm.0.06$	$4.19^{bc}\pm0.03$	$3.27^{d}\pm0.07$	$3.17^{d}\pm0.08$
	Control	$4.86^{a}\pm0.06$	$4.24^{bc}\pm0.03$	$4.00^{a}\pm0.07$	$3.82^{a}\pm0.08$

*Different letters in each column indicate a significant difference ($p<0.05$).

extracts or essential oils, in addition to improving nutritional and qualitative characteristics and increasing the shelf life of meat products, had no significant effect on the consistency and texture of meat products such as sausages. Plant extracts, despite having active ingredients and valuable pigment compounds, lack these two groups of macromolecules and therefore, their effect on consistency will not be as strong as their effects on sausage color and flavor. In conclusion, it can be concluded that treatment containing the rosemary extract (60 ppm) had more effect on the final quality of sausage samples. However, these results may be important due to the presence of different concentrations of blackberry and rosemary extract in the production of lower nitrite products. According to Lynch and Faustman (54), 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. This result conflicted with the findings of Ismail et al. (55) and Nam et al. (56), who reported that the addition of antioxidants has no effect on the lightness of irradiated beef steaks and ground beef during storage. 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 the products (54). Indicating a color change from red to brown possibly due to the formation of metmyoglobin (57). Under a reducing condition, a dark red color is observed due to the formation of nitric oxide myoglobin (58). Lipid oxidation might initiate the oxidation of myoglobin to metmyoglobin, and change the meat color from red to brown. The rate of meat discoloration is closely related to the rate of myoglobin oxidation induced by lipid oxidation (29). Rohlik et al. (59). stated that the addition of antioxidants (Rosemary, Allspice, Nutmeg, Black pepper, Clove, Sandalwood, Cochineal) to dried sausage showed a positive effect on the color stability and elimination of color. The oxidation of lipids has an adverse impact on the sensory properties especially the flavor and color of the meat products (60). The results were similar with Jamwal et al. (51) in chicken meat patties and Nath et al. (52) in chevon patties. Evaporative losses leading to a decline in juiciness, the reduction in mean consistency scores during refrigerated storage might be due to the relative reduction in moisture and juiciness of the product which leads to texture hardening. Similar reports were noticed by Indumathi and Obul Reddy (28), Jamwal et al. (51), and Nath et al. (52). Kant et al. (53) observed that the sausage samples containing mint extract were not significantly different from the control samples in terms of flavor. Khaleghi et al. (21) showed that the use of *Berberis vulgaris* extract in the formulation of sausage indicated the highest score with regard to flavor. Viuda-Martos et al. (38) reported that adding citrus wastewater, thyme, and oregano extracts had no negative effects on the sensory attributes of the cooked sausage. This result can also be interpreted by the previous studies because adding different plant extracts and essences, despite improving the nutritional and qualitative properties and elevating the shelf life of the meat products, has no significant effect on the consistency and texture of the meat products such as sausage. Because the factor influencing this attribute is the presence of macromolecules like proteins and carbohydrates, which increase the water absorption and maintenance and syneresis reduction. Despite having active ingredients and valuable pigments, extracts lack these two groups of macromolecules, so they will not have the same effect on consistency as on the color and flavor of the sausage.

4. Conclusion

This work showed that replacing nitrite with herbal plants extracts is an effective strategy to develop novel and safe meat products. At the same time, lower residual nitrite levels could be achieved with the use of herbal extracts, and marketing these products may consequently reduce consumers' intake of nitrite. The results showed that with increasing storage time, moisture and ash content increased and the fat and protein content and radical scavenging (antioxidant) activity decreased. Sensory scores also decreased with increasing storage time. The control sample had the highest moisture and protein content and sensory scores. In contrast, the highest antioxidant activity belonged to the treatment containing 90 (mg/kg) rosemary extract. In terms of sensory properties, the treatment containing 60 (mg/kg) obtained the highest sensory scores and was selected as optimal treatment during storage.

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