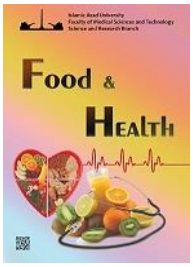

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Evaluation of *Zataria multiflora* and *Salvia leriifolia* extract on the physicochemical and organoleptic characteristics of sausage during refrigerated storage

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ABSTRACT

In the present study, hydroalcoholic extracts of dried and powdered of *Zataria multiflora and Salvia leriifolia* were prepared. After producing the treatments, the moisture, pH, ash, protein and fat content, DPPH radical scavenging activity, color parameters, and sensory evaluation in sausage samples during 45 days of storage were studied. The lowest and highest pH value was related to treatment with the mixture extracts (0.25%) and *Z. multiflora* extract (1.5%), respectively. The lowest and highest protein content was related to 1% *S. leriifolia* and 2% *Z. multiflora* extract, respectively. Gradually, the fat content in the control sample and all treatments has declined, also, the protein and ash content in treatments has decreased and increased respectively. Over time and with increasing the extracts concentration, *L**, *a** and *b** values in all treatments decreased, increased and decreased respectively. *S. leriifolia* extract has a more significant effect on the DPPH test in comparison with *Z. multiflora* extract. Two herbal extracts had a significant effect on the sensorial characteristics of all treatments. It can be said that, in terms of color parameters and free radical scavenging activity, the treatments with *S. leriifolia* extracts were better than control and treatments containing *Z. multiflora* extracts, while, the physicochemical and sensorial properties of recent treatments were better than *S. leriifolia* extracts and control.

1. Introduction

Meat and meat products are susceptible to quality deterioration due to their rich nutritional composition (1). The quality deterioration is due to chemical and microbial changes. The most common form of chemical deterioration is the oxidation of meat lipids. Lipid oxidation is a complex process and depends on the chemical composition of meat, light and oxygen access and storage temperature (2). Extracts from oregano, thyme, rosemary, sage, and mint have been used to improve sensory characteristics (taste, odor, appearance) and extend the shelf life of foods especially meat and meat products such as sausages. Plants extracts and essential oil are bioactive sources with antioxidants, antimicrobial effects and increasing shelf life of products. There is a lot of consumer demand for green food products results in high safety and nutritional values (3). The effects of the plants affected by the type of spice, pH, temperature, and composition of the food model (4). In recent years, there have been many studies on various effects of medical plants in different food models (5). The *Lamiaceae* family is rich in secondary metabolites which have herbal properties and also use in food, cosmetic and sanitary industry. Nouruozak, with the scientific name of *Salvia leriifolia*, is an herbaceous plant belonging to the *Lamiaceae* family and grows in Khorasan and Semnan provinces. Nowadays, various medicinal properties of this plant have been known. Studies have shown that salvia has various medicinal and antimicrobial properties, and its outer skin contains mucilage. The value of the medicinal product of the herb depends on the presence of monoperpropane and di-terpenes, phenolic acid, flavonoids, and antioxidants (6). In recent years, various properties of this plant have proved such as blood glucose-lowering, analgesic, anti-inflammatory, and antioxidant agents. Studies have shown that the leaves and

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roots of *S. leriifolia* have a very strong antioxidant that can compete with common antioxidants in the food industry such as butylhydroxytoluene (BHT) and alpha-tocopherol (7). *Zataria multiflora* is an herb of the *Lamiaceae* family and grows in the central and southern regions of Iran (8). Its Persian name, "Avishane Shirazi" has been traditionally used and prescribed for flavoring and preserving foods (9). *Z. multiflora* essential oil and extract have phenolic (such as thymol, carvacrol, and linalool) and non-phenolic (such as p-cymene, γ-terpinene, and α-pinene) compounds which has antimicrobial, antimicrobial properties (10). Considering the presence of bioactive compounds in plant extracts and their positive effects on the quality of meat products, the quality attributes of sausage with *Zataria multiflora* and *Salvia leriifolia* extract during cold storage was determined.

2. Materials and methods

2.1. Sausage Preparation

The raw materials and common ingredients were used per Kg of meat mixture for the production of sausages as showed in Table 1. Treatments were prepared as follows: A control sample produced without adding herbal extracts. Sausage making was started with the preparation of the material and then the meat (55 %) was ground. Milling was done by using a food processor. The next stage was the addition of 20.58% ice water, 10.5% soybean oil (Behshahr Vegetable Oil Factory, Tehran, Iran), 1.5% salt, 1.5% spices, 0.40% sodium tripolyphosphate, 0.012% sodium nitrite, 0.004% ascorbic acid and non-meat dry ingredients up to a maximum of 10.5% and depending on the extract's ratios (Table 1).

Table 1. The specifications of treatments.

Treatments	*Z. multiflora* extract (%)	*S. leriifolia* extract (%)
T1	1	0
T2	1.5	0
T3	2	0
T4	0	1
T5	0	1.5
T6	0	2
T7	0.25	0.25
T8	0.50	0.50
C	0	0

2.2. Preparation of Plants Extracts

After confirming *Z. multiflora* specified by the Herbarium group of the Research Institute of Forests and Rangelands of Iran (Alborz Province, Karaj), *Z. multiflora* aerial parts were milled after the drying in shade, and transfer to the extraction section (Laboratory of Science and Technology Park of Tehran University, Karaj). For the preparation of the hydroalcoholic extract, 250 g of dried and powdered *Z. multiflora* was macerated with 80% ethanol (80% ethanol–20% water). To obtain the non-polar, semi-polar and polar extract fractions, a powdered sample of *Z. multiflora* was extracted with n-hexane three times during 72 h with constant stirring. The remaining solid material was then extracted with acetone and then with methanol with the same procedure at room temperature (11). The solvents were eliminated from the extracts by evaporation under vacuum in a rotary evaporator. The crude extract and its fractions were stored at -20ºc before experiments. *S. leriifolia* was collected from Sabzevar (Razavi Khorasan province, Northeast of Iran) and specified by the Herbarium group of the Research Institute of Forests and Rangelands of Iran (Alborz Province, Karaj). For the preparation of aqueous extract, the powdered aerial parts (100 g) were boiled in 1000 ml boiling water for 15 min. Subsequently, the mixture was filtered and concentrated under reduced pressure at 35°C (yield: 5.5% w/w). As some constituents are sensitive to boiling water we also prepared a macerated extract. For the preparation of the ethanolic extract, the powdered root (100 g) was macerated in 1000 ml ethanol (96% v/v) for 72 h and subsequently, the mixture was filtered and concentrated under reduced pressure at 35°C (yield: 6% w/w) (12).

2.3. Chemical properties

2.3.1.Determination of pH Value

The pH test was performed according to standard method NO.1028, Institute of Standards and Industrial Research of Iran (ISIRI, 1028/1996). 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).

2.3.2.Determination of Protein Content

At first, about 2 grams of sample were prepared. 20 ml of the 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 (ISIRI, 924/1996) (13).

3.3.2. 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 equation (ISIRI, 742/2002) (14).

2.3.3.*Determination of Ash Content*

At first, 1.5-2 g of the sample was uniformly spread inside the crucible and heated to 20°C in an electric furnace at 550±25°C. It was then cooled in the desiccator at ambient temperature and then weighed by laboratory scale (ISIRI, 744/2003) (15).

2.3.4.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±2ºC. 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 place the plate inside the desiccator to cool. Ultimately weighed (ISIRI, 745/2003) (16).

2.4. DPPH Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was analyzed using the method of Alam et al. (17). The DPPH (Sigma-Aldrich; Milwaukee, USA) solution was prepared by mixing 0.2 mM DPPH in ethanol. The control was determined using 2 ml of distilled water with 2 ml of DPPH solution. The mixture of 2 ml of distilled water with 2 ml of ethanol (Sigma-Aldrich; Milwaukee, USA) was used as a blank of control. The samples used 2 ml of meat supernatant with 2 ml of DPPH solution and the blank of the sample used 2 ml of meat supernatant with 2 ml of ethanol. The decrease in absorbance (Abs) was measured at 517 nm after 30 min. incubation at room temperature in the dark. The percentage of inhibition was calculated as:

$$\text{DPPH radical scavenging activity}(\%)=(A-B)–(C-D)\times100)/(A-B)$$

2.5. Color Parameters

Colorimetry was performed using a colorimeter (Konica Minolta, CM-3500d, Chiyoda, Japan) according to the CIE Lab scale. The instrument was calibrated before use. The color measurement resulted in CIE Lab values for lightness (L*, L=100 is white and L=0 is black), redness (a* red to green component) and yellowness (b*, yellow to blue component). The treatments were placed on a petri dish (Konica Minolta, CM-A128 Petri Dish, Chiyoda, Japan). The sample was illuminated with D65-artificial daylight (10º standard angle) according to the conditions provided by the manufacturer.

2.6. Sensory Evaluation

Sensory evaluation was carried out for uncooked and cooked sausages. Sausages were examined for their color, flavor, consistency and overall acceptance. The recipes were coded with three digits random number. There were 12 panelists in all sensory evaluation tests. Sensory attributes were evaluated using seven points hedonic scale (18), from 1=dislike extremely, 2=dislike very much, 3=dislike, 4=neither like nor dislike, 5=like, 6=like very much and 7=like extremely.

2.7. Statistical Analysis

The experimental design was completely randomized. All analyses and data reported were performed as the mean ± standard deviation. Statistical analysis was performed in SPSS version 24 software for Windows (IBM). Duncan's Multiple Range Test was used to determine the significant differences ($p<0.05$) among group means.

3. Results and Discussion

Moisture, ash, protein and fat content and their changes in samples within 45 days of storage are shown in Table 2. The nutritional component and chemical properties of treatments were affected by *Z. multiflora* and *S. leriifolia* extract. Statistical analysis of the data did show significant differences among the treatments with the control sample ($p<0.05$).

Over time, the pH value of the treatments and the control sample decreased. The lowest and highest of pH value was related to treatment with the mixture extracts (0.25%) and Z. multiflora extract (1.5%), respectively. There was a significant difference between treatments and storage time ($p<0.05$). The use of herbal extracts has a remarkable effect on the pH value of treatments and the pH value declined (Table 2). Emiroglu et al. (19). Reported that the pH values of all fresh ground beef patty samples treated with thyme and oregano extracts generally decreased after the sixth day of storage. Contrary to these results, Mohamed and Mansour (20) reported that no significant differences were observed in the pH values of beef patties after incorporating natural herbal extracts. The reduction in pH value was due to the formation of lactic acid by lactic acid bacteria that consumed the added carbohydrate in the formulation as an energy source (21). The addition of herbal extracts decreased the pH value, which might have helped during the curing process to correct the reduction of nitrite to nitric oxide (22). The reducing pH value might be due to the accumulation of metabolites by bacterial action in meat in addition to protein and amino acid degradation resulting in the formation of ammonia and consequent increase in pH value. The lowest and highest protein content was related to 1% *S. leriifolia* (T4) and 2% *Z. multiflora* (T3) extract, respectively. The protein content in the control sample decreased throughout the storage period. As well as, there was a significant difference between all treatments and storage time ($p<0.05$). The lowest and highest protein content was related to 1% *S. leriifolia* (T4) and 2% *Z. multiflora* (T3) extract, respectively. The protein content in the control sample decreased throughout the storage period. In general, the reduction of proteins over time is related to the oxidation reactions on the sausage proteins. Reducing nutrients such as protein content during cold storage may be due to the protein degradation and the availability of its compounds as a nutrient source for the consumption of all types of bacteria present in it, although the reduction in nutrients in this way is probably

negligible. Over time, the fat content in the control samples and all treatments has declined. In the case of ash, it has also been observed that the ash content increases. The treatments containing the extracts conform to the standard for meat products. Over time, the fat content of the control sample and treatments decreased. In general, the reduction of fat over time is related to the oxidation reactions on the sausage fats. Reducing fat content in treatments in comparison with control of the sample shows that oxidation has occurred in meat fats. The higher concentration of extracts, result in increased ash content. With increasing storage time and extracts concentration, the ash content increased. Viuda-Martos et al. (23) 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 stability during cold storage and light time acting are very important quality attributes of meat products (24).

Table 2. Mean comparison of pH value, protein, fat and ash content of treatments during storage.

Treatment	1st day	15th day	30th day	45th day
		pH value		
C	6.23±0.01 ^{A,a}	6.15±0.03 ^{B,b}	6.09±0.01 ^{C,a}	6.05±0.04 ^{D,b}
T1	6.21±0.01 ^{A,b}	6.18±0.01 ^{B,a}	6.10±0.01 ^{C,a}	6.10±0.01 ^{C,a}
T2	6.23±0.01 ^{A,a}	6.19±0.02 ^{B,a}	6.07±0.03 ^{D,b}	6.11±0.01 ^{C,a}
T3	6.20±0.01 ^{A,b}	6.10±0.00 ^{B,d}	6.07±0.03 ^{C,b}	6.00±0.00 ^{D,d}
T4	6.21±0.02 ^{A,b}	6.14±0.01 ^{B,b}	6.06±0.04 ^{C,b}	6.02±0.03 ^{D,c}
T5	6.19±0.01 ^{A,c}	6.12±0.01 ^{B,c}	6.10±0.00 ^{C,a}	6.01±0.03 $^{D.c}$
T6	6.24±0.04 ^{A,a}	6.11±0.01 ^{B,c}	6.06±0.05 ^{C,b}	6.02±0.03 ^{D,c}
T7	6.18±0.01 ^{A,c}	6.03±0.01 ^{B,e}	6.00±0.00 ^{C,c}	6.00±0.01 ^{C,d}
T8	6.19±0.01 ^{A,c}	6.00±0.00 ^{B,f}	6.00±0.00 ^{B,c}	6.00±0.00 ^{B,d}
		Protein content (%)		
C	20.33±0.22 ^{A,a}	19.96±0.07 ^{B,a}	19.00±0.00 ^{C,b}	18.95±0.07 ^{C,a}
T1	19.67±0.58 ^{B,c}	19.59±0.36 ^{B,b}	19.88±0.12 ^{A,a}	18.46±0.08 ^{C,b}
T2	18.84±0.26 ^{A,e}	18.92±0.13 ^{A,c}	18.89±0.20 ^{A,b}	18.48±0.06 ^{B,b}
T3	20.00±0.00 ^{A,b}	19.62±0.33 ^{C,b}	19.85±0.26 ^{B,a}	18.88±0.10 ^{B,a}
T4	18.71±0.25 ^{A,e}	18.54±0.03 ^{B,d}	18.49±0.16 ^{B,c}	18.04±0.04 ^{C,cd}
T5	19.56±0.38 ^{A,c}	18.64±0.21 ^{B,d}	18.39±0.10 ^{C,c}	18.00±0.00 ^{D,d}
T6	18.62±0.33 ^{B,e}	19.00±0.00 ^{A,c}	19.00±0.00 ^{A,b}	18.21±0.02 ^{C,c}
T7	19.00±0.00 ^{B,d}	19.88±0.20 ^{A,a}	19.92±0.13 ^{A,a}	18.18±0.03 ^{C,c}
T8	19.66±0.30 ^{A,c}	18.88±0.19 ^{B,c}	18.92±0.13 ^{B,b}	17.89±0.11 ^{C,d}
		Fat content (%)		
C	18.00±0.00 ^{A,a}	17.92±0.07 ^{A,a}	17.86±0.16 ^{B,a}	17.38±0.07 ^{C,a}
T1	17.46±0.17 ^{A,bc}	17.37±0.08 ^{A,c}	17.27±0.06 ^{B,c}	17.00±0.00 ^{C,b}
T2	17.67±0.59 ^{B,b}	17.97±0.06 ^{A,a}	17.59±0.36 ^{B,b}	17.07±0.13 ^{C,b}
T3	17.86±0.16 ^{AB,ab}	17.97±0.06 ^{A,a}	17.59±0.35 ^{B,b}	17.44±0.10 ^{C,a}
T4	16.87±0.11 ^{B,d}	17.03±0.06 ^{A,d}	16.94±0.05 ^{A,d}	16.62±0.12 ^{C,c}
T5	17.00±0.00 ^{B,cd}	17.18±0.13 ^{A,cd}	17.09±0.16 ^{A,c}	17.11±0.11 ^{A,b}
T6	17.67±0.35 ^{A,b}	17.61±0.08 ^{A,b}	17.16±0.15 ^{B,c}	17.14±0.09 ^{B,b}
T7	16.92±0.13 ^{A,cd}	16.85±0.35 ^{B,d}	17.03±0.06 ^{A,c}	16.65±0.38 ^{C,c}
T8	16.89±0.10 ^{A,d}	16.14±0.07 ^{C,e}	16.35±0.04 ^{B,e}	16.37±0.06 ^{B,d}
		Ash content (%)		
C	2.47±0.03 ^{D,d}	2.50±0.01 ^{C,d}	2.60±0.01 ^{B,e}	2.63±0.03 ^{A,f}
T1	2.54±0.04 ^{C,b}	2.55±0.01 ^{C,c}	2.58±0.01 ^{B,ef}	2.64±0.03 ^{A,f}
T2	2.57±0.02 ^{C,a}	2.50±0.01 ^{B,d}	2.52±0.03 ^{AB,f}	2.54±0.03 ^{A,g}
T3	2.58±0.03 ^{C,a}	2.60±0.00 ^{BC,b}	2.58±0.03 ^{C,ef}	2.69±0.02 ^{A,e}
T4	2.38±0.01 ^{D,e}	2.41±0.04 ^{C,f}	2.59±0.03 ^{A,e}	2.53±0.02 ^{B,g}
T5	2.57±0.04 ^{C,a}	2.55±0.01 ^{C,c}	2.85±0.04 ^{B,a}	3.00±0.00 ^{A,c}
T6	2.50±0.01 ^{C,c}	2.46±0.02 ^{D,e}	2.74±0.07 ^{B,b}	2.87±0.07 ^{A,d}
T7	2.57±0.03 ^{D,a}	2.64±0.03 ^{C,a}	2.68±0.02 ^{B,c}	3.14±0.05 ^{A,a}
T8	2.46±0.04 ^{C,d}	2.65±0.05 ^{B,a}	2.65±0.04 ^{B,d}	3.10±0.01 ^{A,b}

*Data are expressed as mean ± standard deviation ($n=3$); ** Values within each type of treatment method marked by the same letter within the same column are not significantly different ($p< 0.05$). ***The lower and upper case letters indicate that there is no significant difference in each column or row respectively.

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 (25).

Table 3. The color parameters (L*, a*, b*) of treatments during storage.

Treatment	1st day	15th day	30th day	45th day
		L* value		
C	44.86±0.25 A,a	39.38±0.54 B,b	37.66±0.41 C,a	37.03±0.51 D,a
T1	44.62±0.33 A,a	39.93±0.45 B,a	36.33±0.40 C,d	36.21±0.10 C,c
T2	44.36±0.57 A,b	38.69±0.72 B,c	36.67±0.47 C,c	36.35±0.32 D,bc
T3	44.62±0.35 A,a	38.83±0.40 B,c	36.27±0.65 C,d	36.11±0.15 C,c
T4	43.30±0.61 A,d	39.70±0.52 B,a	37.30±0.33 C,b	36.63±0.32 D,b
T5	43.92±0.68 A,c	36.68±0.29 B,d	35.59±0.39 C,e	35.51±0.43 C,d
T6	43.78±0.38 A,c	38.50±0.87 B,c	35.55±0.39 C,e	35.05±0.20 D,e
T7	44.61±0.41 A,a	39.19±0.11 B,b	35.41±0.53 D,e	36.02±0.29 C,c
T8	42.73±0.28 A,e	37.72±0.24 B,c	35.01±0.23 C,f	35.00±0.00 C,e
		a* value		
C	7.25±0.13 C,c	7.25±0.05 C,cd	7.60±0.09 B,d	8.11±0.01 A,d
T1	7.16±0.05 C,d	7.36±0.03 BC,bc	7.57±0.12 B,d	8.25±0.06 A,c
T2	7.40±0.01 BC,b	7.32±0.07 C,c	7.67±0.03 B,c	8.25±0.13 A,c
T3	7.45±0.04 BC,ab	7.40±0.01 C,b	7.71±0.03 B,c	8.28±0.03 A,bc
T4	7.41±0.07 B,b	7.19±0.02 C,d	7.52±0.01 B,e	8.17±0.06 A,cd
T5	7.37±0.04 C,b	7.47±0.03 C,ab	7.85±0.05 B,ab	8.35±0.08 A,b
T6	7.41±0.03 C,b	7.42±0.11 C,b	7.78±0.23 B,b	8.43±0.04 A,a
T7	7.50±0.04 C,a	7.46±0.13 C,ab	7.92±0.07 B,a	8.44±0.06 A,a
T8	7.49±0.06 C,a	7.53±0.08 C,a	7.80±0.21 B,b	8.35±0.19 A,b
		b* value		
C	18.46±0.32 A,b	18.42±0.90 A,b	18.01±0.23 B,b	17.04±0.40 C,b
T1	18.28±0.21 A,c	18.16±0.19 A,d	17.46±0.27 B,c	17.01±0.23 C,b
T2	18.44±0.28 A,b	18.50±0.50 A,b	18.37±0.43 A,a	17.05±0.57 B,b
T3	19.02±0.29 A,a	18.36±0.19 B,c	18.37±0.70 B,a	17.07±0.12 C,b
T4	18.33±0.25 A,b	18.18±0.28 A,d	18.01±0.23 B,b	17.00±0.00 C,b
T5	19.01±0.23 A,a	19.00±0.00 A,a	18.00±0.00 B,b	17.35±0.43 C,a
T6	19.00±0.00 A,a	19.00±0.00 A,a	18.00±0.00 B,b	17.40±0.13 C,a
T7	18.26±0.61 A,c	17.50±0.55 B,e	17.39±0.51 B,c	17.29±0.30 B,a
T8	18.34±0.15 A,b	17.34±0.46 B,f	17.36±0.38 B,c	17.00±0.00 C,b

*Data are expressed as mean ± standard deviation(n=3); **values within each type of treatment method marked by the same letter within the same column are not significantly different ($p<0.05$).*** The lower and upper case letters indicate that there is no significant difference in each column or row respectively.

Over time and with increasing the concentration of the extract, *L** value in all treatments decreased significantly. The color of *Z. multiflora* and *S. leriifolia* extract resulted in appearing in the brown color (Table 3). The a* value of the treatments with extracts increased significantly compared to the control sample ($p<0.05$). The b* value showed an irregular decrease during storage time. The L* value represented the luminosity, it indicated lightness (26). In the current study, over time and with increasing the concentration of the extract, L* value in all treatments decreased significantly. Murphy et al. (27). similarly reported that the L* value in some recipes of surimi sausage decreased significantly from 71.57 to 55.95 only 12 days after preservation, while for a* and b* values no significant changes were observed. Contrary to these results, Jo et al. (28) reported that the L* value of irradiated pork sausage increased up to 71 during storage whereas for a* value this effect was decreasingly. Akamittath et al. (29) suggested that discoloration and lipid oxidation are interrelated and pigment oxidation may catalyze lipid oxidation. These changes in color are due to protein denaturation and coagulation caused by acid production (30). The biochemical basis of red color in meats is well established and depends on the concentration and redox state of haem pigments in meat (26). The color of meat products is a very important factor to evaluate. The intensity of the meat product red color depends on the concentration of haem pigments such as myoglobin and hemoglobin and is also affected by the intravital conditions of the slaughtered animal and technological processes (31). Haem pigments occurring in the meat products oxidize due to the heat treatment, which can easily react with low molecular substances such as amino acids, nucleotides, etc (32). The a* value of the treatments with extracts increased significantly compared to the control sample ($p<0.05$). Over time, and especially in the last two weeks, the a* value of all treatments increased. Similar results were obtained in other studies (33), in which the a* values of sausages increased during fermentation and decreased during maturation due to dehydration (34). Contrary to these results, according to Lynch and Faustman (35), 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. Also, similarly, Nunez de Gonzalez et al. (36) reported that a* value of beef stored under refrigeration for 10 weeks decreased significantly. Khaleghi et al. (37) studied the 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 and reported that samples containing the extract had similar redness but lower

lightness when compared to the control sausage sample. A decrease in a* values corresponding to the decreased redness of lamb meat as a result of myoglobin oxidation (met-myoglobin formation) has been reported previously (38, 39) This result conflicted with the findings of Ismail et al. (40) and Nam and Ahn (41), 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 (35). Indicating a color change from red to brown possibly due to the formation of metmyoglobin (42). Under a reducing condition, a dark red color is observed due to the formation of nitric oxide myoglobin (43). 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 (21, 44). Rohlik et al. (31) 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. These color changes (decrease of redness a*) in cuts of dry sausages exposed to air and the light are influenced by the concentration of rosemary oleoresin, mostly by its light fraction.

Table 4. DPPH scavenging activity at 50 µl/mg of extracts during storage.

Treatments	1st day	15th day	30th day	45th day
C	19.00±0.00 ^{B,e}	17.92±0.12 ^{C,g}	19.67±0.58 ^{A,c}	6.20±0.18 ^{D,i}
T1	25.82±0.17 ^{B,a}	41.00±0.00 ^{A,a}	22.82±0.17 ^{C,a}	13.73±0.35 ^{D,a}
T2	21.88±1.02 ^{B,b}	24.22±1.07 ^{A,c}	20.71±0.55 ^{C,b}	12.72±0.25 ^{D,b}
T3	18.55±0.51 ^{A,f}	18.72±0.25 ^{A,f}	17.16±0.24 ^{B,e}	10.00±0.00 ^{C,c}
T4	17.03±0.46 ^{B,g}	17.02±0.34 ^{B,h}	17.62±0.33 ^{A,de}	7.01±0.23 ^{B,g}
T5	16.18±0.31 ^{C,i}	17.63±0.34 ^{B,gh}	17.96±0.75 ^{A,d}	9.33±0.58 ^{D,d}
T6	20.33±0.58 ^{C,c}	22.81±0.18 ^{A,d}	20.67±0.58 ^{B,b}	6.55±0.51 ^{D,h}
T7	19.55±0.51 ^{B,d}	19.77±0.23 ^{A,e}	19.62±0.33 ^{A,c}	8.97±1.05 ^{C,e}
T8	16.70±0.60 ^{C,h}	31.55±0.51 ^{A,b}	22.82±0.32 ^{B,a}	8.07±0.11 ^{D,f}

*Data are expressed as mean ± standard deviation (n=3); **values within each type of treatment method marked by the same letter within the same column are not significantly different ($p< 0.05$). ***The lower and upper-case letters indicate that there is no significant difference in each column or row respectively.

In his study, the b* value showed an irregular decrease during storage time. Reducing the yellowness in different concentrations of the extract can be attributed to the interaction of aromatic compounds in essential oil and extract with nitrite in the sausage. Lauritzen and Martinsen (45) 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. (46) reported that lipid oxidation correlated with an increase in b* values in cooked turkey products during refrigerated storage. The yellow pigment in the meat product is produced by the nonenzymatic browning reactions occurring between lipid oxidation products and amino groups of the proteins. Murphy et al. (27) reported that in some recipes of surimi sausage, the b* values no significant changes were observed. The changes in b* found during fermentation and maturation in the present study were also found in another study (47) on salami sausages, and they are probably due to the oxygen consumption by microorganisms during their exponential growth phase and the decrease in oxymyoglobin, which contributes to the b* values. The DPPH radical scavenging activity of treatments are displayed in Table 4. With increasing the concentration of *Z. multiflora* extract, radical scavenging activity decreases. The antioxidant activity promotes increasing the concentration of *S. leriifolia* extract. The results of the treatment mixture showed that, with the increasing of both extracts, the scavenging activity increased. As a result of this, it could be concluded that all treatments contained antioxidants higher than the control sample. Over time, the sensory scores of treatments were declined. The manifested antioxidant effect of the tested extracts in sausages was considered significant because 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 (48). The DPPH radical scavenging activity in treatments was higher than the control sample, which contained several compounds such as polyphenolics, flavonoids, lignans, and terpenoids in *Z. multiflora* and *S. leriifolia* extract (Table 4). This result has been reported in Rather et al. (49) study. Also, Coutinho de Oliveira et al. (50) 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 (51). With increasing of equal extracts (0.25, 0.50 %) in treatments, the free radical scavenging activity increased. In contradictory, according to previous studies (52), results showed a relationship between the concentration of individual extracts,

although, there was no linear relation established between an increase in the concentration of one extract in combination and synergism effect. These results suggest that the addition of Z. *multiflora* and *S. leriifolia* extract had retarded the lipid oxidation during the storage. Also, these results agree with that reported by Mc Carthy et al. (34) and Sebranek et al. (53). Karpinska et al. (54) reported that application of 1.5% of sage alone was more effective on turkey meat dish storage stability than the 1% mixture of spices (sage, red pepper, black pepper, garlic, and marjoram). The quality of products with 1.5% sage was good after a-four-day storage in the refrigerator. Contrary to these results, Simitzis et al. (55) 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 (56). In the current study, the sensory scores of treatments were declined during cold storage (Fig. 1). In terms of overall acceptance, the treatments with *Z. multiflora* extract had a higher score). The oxidation of lipids has an adverse impact on the sensory properties especially the flavor and color of the meat products (32). The results were similar to Jamwal et al. (57) in chicken meat patties and Nath et al. (58) 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. In terms of color, in the last two weeks, there was a significant difference between the control sample and the treatments ($p<0.05$). The highest score among the treatments on the final day was related to treatment with 1% *S. leriifolia* extract. There was a significant difference between the flavor scores of the control sample in comparison with all treatments ($p<0.05$) except the treatment with 1.5% of *Z. multiflora* extract, which had the highest score among the treatments.

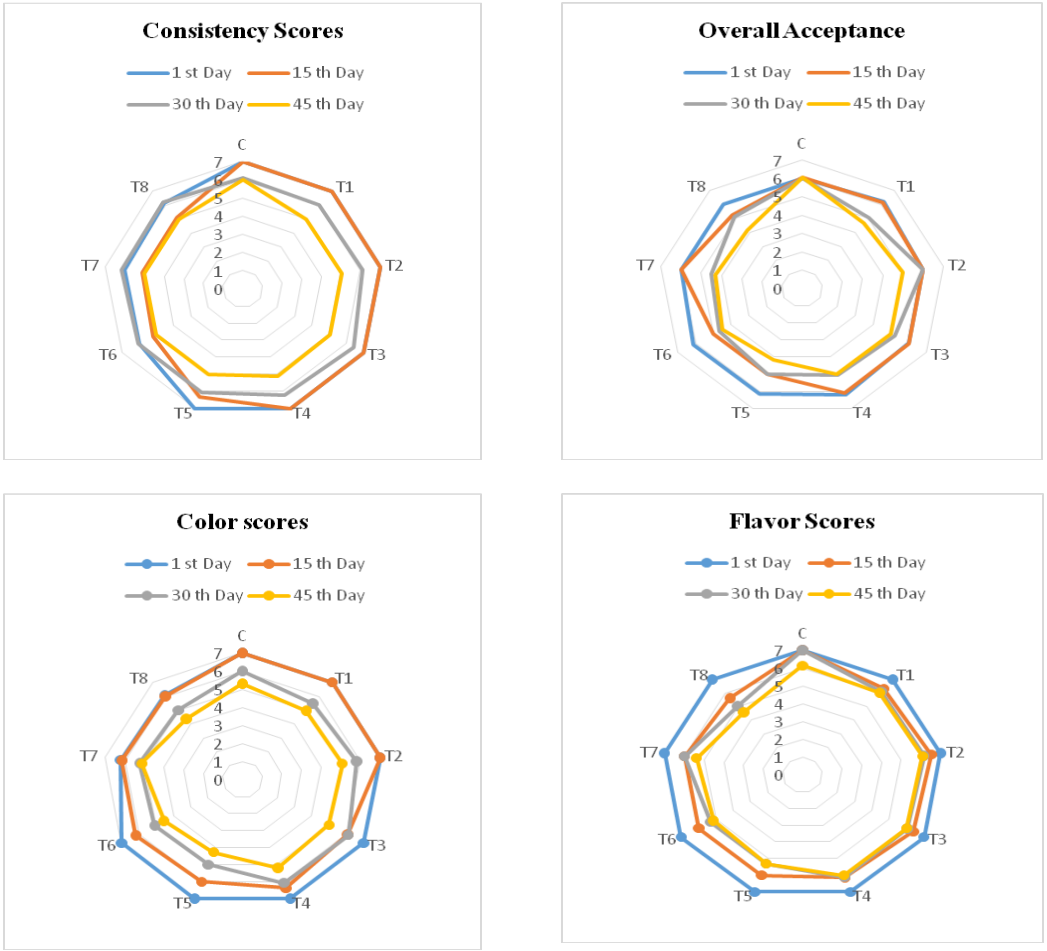

Fig. 1. Sensory evaluation scores for treatments during storage. *1=dislike extremely, 2=dislike very much, 3=dislike, 4=neither like nor dislike, 5=like, 6=like very much and 7=like extremely. **Means within the same column with different letters are significantly different ($p<0.05$). *** All values were expressed as mean ± standard deviation.

The lowest flavor score belonged to the treatment with equal concentrations of both extracts (0.5%), which had a significant difference with the control sample and other treatments. The treatment with equal concentrations of both extracts (0.25%)

has the lowest consistency score during refrigerated storage. At the end of the storage period, the highest and lowest consistency scores were related to treatment with 1% *S. leriifolia* extract and 1% *Z. multiflora* extract. In terms of overall acceptance, the treatments with *Z. multiflora* extract had a higher score. The lowest score belonged to the treatment with 1.5% of the S. leriifolia extract, which had a significant difference with the control sample ($p<0.05$). 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 oxidation of lipids has an adverse impact on the sensory properties especially the flavor and color of the meat products (32). The results were similar to Jamwal et al. (57) in chicken meat patties and Nath et al. (58) 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. Similar reports were noticed by Indumathi and Obul Reddy (59), Jamwal, et al. (57) and Nath et al. (58).

4. Conclusion

The nutritional component and chemical properties of treatments were affected by *Z. multiflora* and *S. leriifolia* extract. The lowest and highest of pH value was related to treatment with the mixture extracts (0.25%) and *Z. multiflora* extract (1.5 %) respectively. The highest protein content was related to 2% *Z. multiflora* (T3) extract. Over time, the fat and protein content has declined, while, the ash content has increased. With increasing the extracts concentration, L*, a* and b* values in all treatments decreased, increased and decreased respectively. *S. leriifolia* extract has a more significant effect on the free radical scavenging activity in comparison with *Z. multiflora* extract. In terms of sensorial characteristics, the treatments with *Z. multiflora* extract had a higher score. It can be said that, in terms of color parameters (L*a*b*) and free radical scavenging activity, the treatments with *S. leriifolia* extracts were better than control and treatments containing *Z. multiflora* extracts, while, the physicochemical and sensorial properties of recent treatments were better than *S. leriifolia* extracts and control.

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