

Effect of *Carum copticum* L. and *Salvia officinalis* L. Extracts on the Physicochemical, Microbial, and Sensory Characteristics of Heat -Treated Sausage during Refrigerated Storage

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ABSTRACT

In the present research, 10, 20, and 30 mg kg⁻¹ of *Carum copticum* extract, 40, 50, and 60 mg kg⁻¹ of *Salvia officinalis* extract, and a combination of these extracts (15:30) were used as partial replacements of nitrite in the sausage formulation. Then, the physicochemical, microbial, and sensory tests were performed on all treatments and control sample (without any extract) over 45 days. The results showed that treatment with 60 mg kg⁻¹ *S. officinalis* extract had the lowest pH value. The lowest peroxide value was related to treatment with 40 mg kg⁻¹ *S. officinalis* extract, which showed a significant difference from the control sample ($P < 0.05$). The highest microbial count belonged to the control sample. Treatment with 60 mg kg⁻¹ *S. officinalis* extract had the least total bacterial and *Clostridium perfringens* count. In contrast, the lowest count of coliform, mold, and yeast was found in treatment with 40 mg kg⁻¹ *S. officinalis* extract. In terms of sensory attributes, treatment with 30 mg kg⁻¹ *C. copticum* extract had the highest color and flavor scores, while the highest consistency and overall acceptance scores for all treatments were related to 20 and 10 mg kg⁻¹ *C. copticum* extract, respectively. The results of this study showed that certain concentrations of *C. copticum* and *S. officinalis* extracts should be selected to achieve the optimal qualitative properties of sausage. In general, treatment with various concentrations of *S. officinalis* extract had better acceptance and improved the quality of the sausages. In conclusion, treatment containing 40 mg kg⁻¹ *S. officinalis* extract was selected as a better treatment due to physicochemical, microbial, and sensory characteristics during cold storage.

Keywords: Herbal extract, Microbial count, Nitrite content, Sausage quality.

INTRODUCTION

Plants have been one of the important sources of medicines ever since the dawn of human civilization. Chemically, medicinal plants may have secondary metabolites like alkaloids, glycosides, steroids or other groups of compounds that have marked pharmaceutical action as anticancer,

antimalarial, antidiabetic, antidiysenteric, etc. Herbal plants have been the traditional source for raw material and finished herbal drugs since ancient times. The usage of medicinal plants as preventive and curative medicines is sufficiently documented in many scholastic works (Nagaraju, 2016). Food processors and consumers have expressed a desire to reduce the use of

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synthetic chemicals in food preservation. Recently, there has been considerable interest in extracts and Essential Oils (EOs) from common culinary herbs, spices, and aromatic plants characterized by a notable antimicrobial activity (Hayouni *et al.*, 2008), and partial replacement of nitrite and increase in shelf life of meat and meat products (Taghvaei and Jafari, 2015). Herbal or medicinal extracts have been considered as components with health-promoting ingredients, antioxidant and antimicrobial effects. There is a demand for natural preservatives derived from herbal plants, animal and microbial resources which improve the human health. Furthermore, the World Health Organization (WHO) has recently called for a worldwide reduction in the consumption of salt in order to reduce the incidence of cardio-vascular disease. Numerous studies have documented the beneficial properties of different plants extracts (Burt, 2004). Herbal plants are essentially flavoring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties. Nowadays, plenty of spices and herbs are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities (Shan *et al.*, 2007).

The genus *C. copticum* is a herb of the Apiaceae family that grows in Iran, India, Pakistan and Egypt. In Iranian native medicine, the fruit of *C. copticum* is used as an anti-inflammatory and diuretic agent. This plant contains different important components such as carbohydrates, glucosides, saponins and phenolic compounds (carvacrol), volatile oils (thymol), terpiene, paracymene and beta-pinene, protein, fat, fiber, and minerals including calcium, phosphorus, iron, and nicotinic acid (niacin) (Boskabady *et al.*, 2014). Different therapeutic applications for *C. copticum* have been described and, in Persian traditional medicine, it has been used for thousands of years (Zarshenas *et al.*, 2013). Overall, according to many prior studies, the *C. copticum* EO exhibits potent

antimicrobial and antioxidant activities supporting its potential use for perishable and high fatty foods (Fazeli-nasab and Fooladvand, 2016). Antimicrobial activity of *C. copticum*, which could be attributed to high content of compounds such as thymol, has been reported by large numbers of studies (Oskuee *et al.*, 2011).

Salvia, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world. Some members of this genus are of economic importance since they have been used as flavoring agents in perfumery and cosmetics (Ahmadi and Mirza, 1999). Sage (*Salvia officinalis* L.) grows in the East-Mediterranean region and it belongs to the Lamiaceae family. The plants belonging to the Lamiaceae family grow in many parts of the world, some of them are used to improve the products quality (Lemle, 2018). *S. officinalis* has active compounds such as triene cinnamal boraneol flavonoid saponin, vitamin C and E, tannin, resin, di-terpen, anti-inflammatory, anti-fungal, antimicrobial and antioxidant properties and is used in the treatment of liver and kidney disorders (Ahmadi *et al.*, 2013).

Furthermore, antimicrobial activities of the EOs are difficult to correlate to a specific compound due to their complexity and variability. Nevertheless, some researchers reported that there is a relationship between the chemical composition of the most abundant components in the EO and the antimicrobial activity (Deans and Sbdova, 1990). For example, 1,8-cineole (herein, abundant in *S. officinalis* EO) and camphor are well-known chemicals having antimicrobial potentials (Pattnaik *et al.*, 1997).

(Sojic *et al.*, 2017) and Velasco and Williams (2011) investigated the effect of *S. officinalis* extract on the qualitative properties of pork sausage and meat, respectively. Also, in study by (Zhang *et al.* 2013), during storage of refrigerated Chinese-style sausage formulated with ground sage, a reduction in TBARs values and textural deterioration was observed and

there was no negative effects on the sensory properties. The present study aimed to study the effect of *C. copticum* L. and *S. officinalis* extracts on the physicochemical, microbial, and sensorial characteristics of non-fermented sausage during refrigerated storage.

MATERIALS AND METHODS

C. copticum L. and *S. officinalis* were confirmed by the Herbarium group of the Research Institute of Forests and Rangelands of Iran (Alborz Province, Karaj).

Extraction method

Extraction of *C. copticum*

The method of (Mansouri *et al.*, 2005) was used for extraction, with slight modification. First, 40 g of *C. copticum* seeds were milled and mixed with 100 mL 99.9% acetone solvent and 100 mL water (plant/solvent ratio of 1:5) for 12 hours at 40°C. The samples were stirred every two hours. After extraction, the extracts were filtered with a filter paper (Whatman No 1). The filtered liquid was then concentrated by a rotary evaporator at 40°C until complete withdrawal of the extraction solvent.

Extraction of *S. officinalis*

Extraction of *S. officinalis* leaves was performed by maceration. First, 50 g of dried leaves of the plant was weighed by a digital scale (Lib ROR AEU-210). The leaves were then powdered and transferred to an Erlenmeyer flask. Then, 1,500 cc of the solvent (50% ethanol and 50% water) was added to it such that it covered all the powder. After covering the Erlenmeyer flask with an aluminum foil, it was placed on a shaker (Heidolph Unimax 2010) at 90 rpm. After homogenization of the solvent and powdered plant, the solution was filtered by

a filter paper (Whatman 0.5 mm, USA). The solution was then placed in a rotary evaporator (Heidolph WD 2000) to isolate the solvent from the extract. The pure extract obtained was stored in sterile vials in refrigerator until the relevant tests were performed (Kermanshahi *et al.*, 2009).

Production of Sausage Samples

The overall formulation included 60% meat (Morgh Co.) and other ingredients, including 18.51% water and ice, 14% soybean oil (Behshahr Co.), 1.5% salt, 2.8% starch, 1.8% soy isolate, 0.4% sodium phosphate (Beka Co.), 0.05% ascorbic acid (Shandongluwei Co., China), and 0.9% different spices (Shimi Razan Co.). All ingredients were mixed in a cutter (Laska, Germany) and the obtained paste was mixed with nitrite (BASF, Germany), extract, and half of the remaining water and ice in separate batches in the cutter. All treatments were separately packed in polyamide packages and cooked for 1 hour at 75°C. Then, the product was cooled under a cold shower, transferred to refrigerator at 4°C, and kept for 45 days. The characteristics of the treatments are presented in Table 1.

Physico-Chemical Measurements

pH value

The pH test was performed according to the national standards of the Institute of Standards and Industrial Research of Iran, No 1027.

Cooking loss

The cooking loss test was conducted based on the method proposed by (Hayes *et al.* 2013). The sausage samples were placed in the oven (Mettler, D91126, Germany). After the internal temperature of the samples reached 71°C, they were kept in the oven for

**Table 1.** Characteristics of studied treatments.

Treatment	<i>C. copticum</i> extract (mg kg ⁻¹)	<i>S. officinalis</i> extract (mg kg ⁻¹)	Nitrite (mg kg ⁻¹)
1	10	0	30
2	20	0	30
3	30	0	30
4	0	40	30
5	0	50	30
6	0	60	30
7	15	30	20
C	0	0	120

3 minutes. The cooking loss test was performed based on the weight difference of sausage before and after cooking by the following formula:

$$\text{Cooling loss} = (\text{Raw weight} - \text{Cooked weight}) \times 100 \quad (1)$$

Peroxide value

The measurement of peroxide value of the sausage samples was done by titration method with sodium thiosulphate (0.01N) in the vicinity of 1% reagent starch (AOAC, 2000).

Microbial Tests

The microbial tests performed on the sausage samples included total microbial count (aerobic mesophilic bacteria) by mixed-culture method using plate count agar (national standard 9263, 2007), coliforms count by mixed-culture method using Violet Red Bile Agar (VRBA) (National Standard 9263, 2007), *Cl. perfringens* count using cycloserine medium (National Standard 2197,1992), and molds and yeasts count using Dichloran Rose Bengal Chloramphenicol Agar (National Standard 1-10899, 2008).

Sensory Evaluation

The sausage samples produced were assessed by ten 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 a foil and placed in the oven for 8 minutes. They were then removed from the oven and cut into 2-cm-thick pieces after reaching the ambient temperature. The attributes evaluated consisted of color, flavor, consistency, and overall acceptance of the samples (Iranian National Standard, 2008).

Statistical Analysis

Data analysis was carried out by SPSS-24 software in a complete random design using Duncan's test for comparison of the mean indices in each treatment. $P < 0.05$ was considered significant for all tests. Excel software (2010) was used for drawing the tables and figures. Three replicates were prepared for the processing of each treatment and averaged for the statistical analyses.

RESULTS

Physico-Chemical Measurements

In this study, the lowest pH value was reported on the last day. The treatments with 10 and 20 mg kg⁻¹ *C. copticum* extract were

not significantly different from the control sample ($P > 0.05$). However, the control sample showed a significant difference compared to other treatments ($P < 0.05$). On the last day, there was a significant difference between all treatments and the control sample ($P < 0.05$). The minimum and maximum cooking loss rates on day 45 were found for 30 mg kg⁻¹ *C. copticum* and 60 mg kg⁻¹ *S. officinalis*, respectively (Table 2).

The combination of both extracts showed a higher peroxide value than the extracts used alone, which can be due to an

antagonist relationship between the two extracts regarding their effects on peroxide value. Adding higher concentrations not only did not increase the antioxidant activity but also exerted peroxidant effect, which was fully evident at 2% concentration. During the first month, treatment with *C. copticum* extract indicated lower peroxide value than control samples and treatment with *S. officinalis* extract. But on the final day, the lowest peroxide value was reported for the treatment with 40 mg kg⁻¹ *S. officinalis* extract, which showed no significant difference from treatment with 50

Table 2. Effect of different concentrations of *C. copticum* and *S. officinalis* extracts on pH value, cooking loss (%), and peroxide value (mEq kg⁻¹) in the sausage samples during refrigerated storage. ^a

Treatments	Day 1	Day 15	Day 30	Day 45
pH level				
1	6.20±0.00 ^{C,b}	6.11±0.00 ^{C,c}	6.09±0.02 ^{B,a}	6.03±0.01 ^{A,a}
2	6.22±0.00 ^{C,b}	6.14±0.00 ^{C,c}	6.08±0.01 ^{B,a}	6.02±0.04 ^{A,a}
3	6.25±0.00 ^{C,b}	6.14±0.00 ^{C,c}	6.02±0.05 ^{B,c}	6.00±0.04 ^{A,b}
4	6.24±0.00 ^{C,b}	6.14±0.00 ^{C,c}	6.04±0.01 ^{A,b}	6.00±0.04 ^{B,b}
5	6.23±0.00 ^{B,b}	6.19±0.01 ^{A,a}	6.04±0.01 ^{A,b}	5.82±0.00 ^{B,d}
6	6.25±0.00 ^{A,b}	6.14±0.00 ^{A,c}	6.01±0.00 ^{A,d}	5.80±0.01 ^{A,d}
7	6.32±0.00 ^{A,b}	6.15±0.00 ^{A,c}	6.00±0.00 ^{A,d}	5.96±0.01 ^{A,c}
C	6.28±0.09 ^{A,a}	6.17±0.06 ^{A,b}	6.04±0.01 ^{A,b}	6.04±0.00 ^{B,a}
Cooking loss (%)				
1	4.28±0.07 ^{C,b}	6.00±0.00 ^{D,b}	14.03±0.05 ^{B,a}	16.11±0.20 ^{A,b}
2	4.72±0.04 ^{C,a}	6.03±0.01 ^{D,b}	12.72±0.06 ^{B,b}	15.16±0.25 ^{A,b}
3	4.26±0.07 ^{D,b}	5.49±0.09 ^{C,c}	9.27±0.21 ^{B,d}	12.89±0.89 ^{A,d}
4	4.05±0.05 ^{C,d}	5.02±0.02 ^{C,d}	10.30±0.06 ^{B,c}	14.44±0.38 ^{A,c}
5	4.07±0.12 ^{C,d}	5.00±0.00 ^{C,d}	11.00±0.00 ^{B,c}	14.04±0.03 ^{A,c}
6	4.48±0.11 ^{C,d}	7.00±0.00 ^{D,a}	15.52±0.42 ^{B,a}	17.11±0.00 ^{A,a}
7	4.62±0.10 ^{C,b}	7.97±0.06 ^{C,a}	15.18±0.06 ^{B,a}	16.57±0.13 ^{A,a}
C	4.33±0.18 ^{D,a}	4.33±0.10 ^{C,e}	7.97±0.06 ^{B,e}	10.15±0.28 ^{A,e}
Peroxide value (mEq.kg)				
1	0.9±0.02 ^{D,a}	1.00±0.05 ^{D,b}	2.60±0.67 ^{D,bc}	2.95±0.67 ^{D,a}
2	0.7±0.04 ^{D,b}	1.00±0.05 ^{D,b}	2.58±0.01 ^{D,c}	2.88±0.13 ^{D,b}
3	0.7±0.04 ^{D,b}	0.9±0.11 ^{D,c}	2.43±0.09 ^{D,d}	2.63±0.09 ^{D,d}
4	0.8±0.04 ^{D,b}	1.01±0.05 ^{D,b}	2.42±0.09 ^{D,d}	2.60±0.09 ^{D,d}
5	0.8±0.04 ^{D,b}	1.00±0.05 ^{D,b}	2.50±0.34 ^{D,c}	2.79±0.34 ^{D,c}
6	0.9±0.02 ^{D,a}	1.00±0.05 ^{D,b}	2.77±0.18 ^{D,b}	2.98±0.18 ^{D,a}
7	0.9±0.02 ^{D,a}	1.13±0.05 ^{D,a}	2.82±0.01 ^{D,a}	2.97±0.01 ^{D,a}
C	1.0±0.02 ^{D,a}	1.00±0.68 ^{D,b}	2.58±0.01 ^{D,c}	2.90±0.01 ^{D,b}

^a (A-D) and (a-e): Data are expressed as mean±standard deviation (n= 3). Values within each type of treatment method marked by the same letter within 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.



mg kg⁻¹ *S. officinalis* extract ($P > 0.05$) but indicated a significant difference from other treatments and the control sample ($P < 0.05$). The highest peroxide value was found for treatment with 60 mg kg⁻¹ *S. officinalis* extract, indicating no significant difference from treatment with mixed *S. officinalis* and *C. copticum* ($P > 0.05$) (Table 2).

Microbial Tests

The changes of total bacterial count from day 1 to 45 were ascending, but this increasing trend was slower in treatments with extracts, especially *S. officinalis* versus *C. copticum* extract and control sample. On day 45, the highest and the lowest total bacterial counts were found for the control sample and treatment with 40 mg kg⁻¹ *S. officinalis* extract, which showed a significant difference ($P < 0.05$) (Table 3).

As for coliform bacterial count, no bacterium was able to grow in all treatments from days 1 to 15. On day 30, the number of grown colonies was less than 5 colonies (Table 4).

On the final day, the treatment with 40 mg kg⁻¹ *S. officinalis* extract showed the least total coliforms count, indicating a significant difference from the control sample ($P < 0.05$). As for the count of coliform bacteria, no bacterial growth was seen in the

treatments from day 1 to 15. On the last day, treatment with 40 mg kg⁻¹ *S. officinalis* extract showed the least coliforms count, which was significantly different from the control sample ($P < 0.05$) (Table 4). Regarding *Cl. perfringens* count, no bacterial growth was seen in the treatments from day 1 to day 15. During days 30 and 45, the mean bacterial count was significantly lower in treatment with *S. officinalis* extract than in treatment with *C. copticum* extract and control sample. On the 30th and 45th days, the control sample had the highest bacterial count, showing a significant difference from all treatments ($P < 0.05$) (Table 4).

During days 1-15, the molds and yeasts of the sausage samples were not countable. On days 30-45, the molds and yeasts count was less than the standard level as well as the acceptable level according to the Institute of Standards and Industrial Research of Iran (100 per g) (Table 4). During days 1-15, the yeasts and molds were not countable in the sausage sample. The control sample showed the highest count for the yeasts and molds, while the least count was found for treatment with 40 mg kg⁻¹ *S. officinalis* extract. In the sausage samples produced in the current study, the antimicrobial compounds of *S. officinalis* and *C. copticum* extracts and damage to microorganisms caused by cooking heat reduced the

Table 3. Effect of different concentrations of *C. copticum* and *S. officinalis* extracts on total bacterial count (log cfu/g) in the sausage samples during refrigerated storage. ^a

Treatments	Total bacterial count (log cfu/g)			
	Day 1	Day 15	Day 30	Day 45
1	1.10±0.08 ^{C,b}	1.59±0.11 ^{C,b}	1.62±0.09 ^{C,c}	1.33±0.08 ^{C,c}
2	1.02±0.17 ^{C,c}	1.76±0.05 ^{A,a}	1.77±0.05 ^{A,b}	2.40±0.44 ^{C,a}
3	1.23±0.08 ^{C,b}	1.74±0.13 ^{A,a}	1.79±0.13 ^{A,b}	2.37±0.08 ^{C,c}
4	1.11±0.08 ^{C,b}	1.43±0.08 ^{C,bc}	1.43±0.15 ^{C,c}	2.00±0.23 ^{C,e}
5	1.43±0.08 ^{C,a}	1.50±0.08 ^{B,b}	1.54±0.15 ^{B,d}	2.14±0.11 ^{C,d}
6	1.30±0.29 ^{C,b}	0.40±0.00 ^{C,bc}	1.40±0.15 ^{C,c}	1.89±0.67 ^{C,ef}
7	1.17±0.08 ^{C,b}	1.50±0.44 ^{B,b}	1.52±0.15 ^{B,d}	2.14±0.12 ^{C,d}
C	1.13±0.08 ^{C,b}	1.80±0.08 ^{A,a}	1.84±0.11 ^{A,a}	2.46±0.00 ^{C,a}

^a (A-D) and (a-e): Data are expressed as mean±standard deviation (n=3). Values within each type of treatment method marked by the same letter within 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.

Table 4. Effect of different concentrations of *C. copticum* and *S. officinalis* extracts coliform, *Cl. perfringens*, mold and yeasts count (log cfu/g) in the sausage samples during refrigerated storage.^a

Treatments	Coliform count (log cfu/g)	
	Day 30	Day 45
1	3±0.04 ^b	5±0.01 ^b
2	4±0.04 ^a	9±0.12 ^a
3	4±0.04 ^a	9±0.12 ^a
4	1±0.11 ^d	3±0.04 ^{cd}
5	2±0.34 ^c	4±0.01 ^b
6	3±0.30 ^b	4±0.01 ^b
7	4±0.04 ^a	9±0.12 ^a
C	4±0.04 ^a	10±0.12 ^a
	Clostridium perfringens count (log cfu/g)	
	Day 30	Day 45
1	26±0.05 ^b	37±0.12 ^c
2	26±0.05 ^b	40±0.12 ^b
3	28±0.05 ^b	43±0.12 ^b
4	19±0.11 ^c	33±0.18 ^c
5	14±0.16 ^d	22±0.07 ^d
6	9±0.42 ^e	17±0.03 ^e
7	26±0.05 ^b	40±0.12 ^b
C	33±0.09 ^a	46±0.01 ^a
	Mold and yeast count (log cfu/g)	
	Day 45	
1	60	
2	76	
3	83	
4	44	
5	70	
6	60	
7	66	
C	87	

^a (a-e): Data are expressed as mean±standard deviation (n= 3). Values within each type of treatment method marked by the same letter within 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.

microbial load. As a result of these findings, the higher antimicrobial activities of *S. officinalis* extract could be attributed to its particular chemotype characterized by its complexity with oxygenated-hydrocarbons as dominant components and the presence of equivalent amounts of monoterpene hydrocarbons and sesquiterpene hydrocarbons (with eucalyptol, α/β -thujone and borneol as major components).

Sensory Tests

Over time, especially in the final month, the sensory scores of the treatments were reduced. In fact, the sensory scores were higher for the treatment with *C. copticum* extract than in the treatment with *S. officinalis* extract. During the storage time, the color scores of treatments with *C. copticum* extract were higher than the control sample and treatment with *S. officinalis* extract. In the final day, the highest score were reported for treatment with 30 mg kg⁻¹ *C. copticum* extract, which was significantly different from other treatments and the control sample (P< 0.05).



The lowest color score on the final day was observed for treatment containing both herbal extracts (Table 5).

With longer storage time, the flavor scores of the treatments decreased. During the storage time, the color scores of treatments

with *C. copticum* extract were higher than the control sample and treatments with *S. officinalis* extract. The lowest flavor score on the final day was found for treatment with the mixture of both extracts, which showed no significant difference from

Table 5. Effect of different concentrations of *C. copticum* and *S. officinalis* extracts on sensory scores in the sausage samples during refrigerated storage. ^a

Treatments	Day 1	Day 15	Day 30	Day 45
Color scores				
1	5.00±0.11 ^{A,a}	4.50±0.58 ^{B,b}	3.70±0.00 ^{C,b}	3.38±0.00 ^{D,d}
2	5.00±0.00 ^{A,a}	4.55±0.00 ^{A,b}	3.77±0.10 ^{B,b}	3.54±0.02 ^{B,c}
3	5.00±0.00 ^{A,a}	5.00±0.00 ^{A,a}	4.00±0.10 ^{B,a}	3.70±0.00 ^{C,b}
4	4.67±0.19 ^{A,b}	4.12±0.00 ^{B,d}	3.51±0.07 ^{B,bc}	3.18±0.00 ^{B,de}
5	4.00±0.00 ^{A,c}	4.33±0.06 ^{B,c}	3.60±0.07 ^{B,bc}	3.36±0.02 ^{B,d}
6	4.00±0.00 ^{A,c}	4.00±0.00 ^{B,d}	3.92±0.06 ^{B,a}	3.04±0.18 ^{C,de}
7	4.00±0.00 ^{A,c}	4.05±0.00 ^{B,d}	3.92±0.06 ^{B,a}	3.10±0.18 ^{C,de}
C	5.00±0.00 ^{A,a}	5.00±0.00 ^{B,a}	4.13±0.00 ^{B,a}	3.88±0.20 ^{C,a}
Flavor scores				
1	5.00±0.11 ^{A,a}	4.67±0.13 ^{B,a}	4.12±0.01 ^{C,b}	3.60±0.11 ^{D,b}
2	5.00±0.11 ^{A,a}	4.00±0.44 ^{A,c}	4.33±0.01 ^{B,b}	3.69±0.11 ^{B,b}
3	5.00±0.11 ^{A,a}	4.50±0.00 ^{A,b}	4.40±0.13 ^{B,a}	4.00±0.20 ^{C,a}
4	5.00±0.19 ^{A,a}	4.00±0.44 ^{B,c}	3.95±0.07 ^{B,c}	3.12±0.30 ^{B,e}
5	5.00±0.00 ^{A,a}	4.01±0.44 ^{B,c}	3.97±0.07 ^{B,c}	3.33±0.00 ^{B,bc}
6	4.66±0.01 ^{A,b}	4.16±0.09 ^{B,bc}	3.92±0.07 ^{B,c}	3.00±0.18 ^{C,c}
7	4.60±0.01 ^{A,b}	4.20±0.13 ^{B,bc}	3.90±0.07 ^{B,c}	3.00±0.18 ^{C,c}
C	5.00±0.11 ^{A,a}	5.00±0.13 ^{B,a}	4.54±0.13 ^{B,a}	4.00±0.20 ^{C,a}
Consistency scores				
1	5.00±0.01 ^{A,a}	4.67±0.71 ^{B,a}	4.00±0.18 ^{C,c}	3.70±0.23 ^{D,b}
2	5.00±0.01 ^{A,a}	4.78±0.71 ^{A,a}	4.48±0.34 ^{B,b}	4.00±0.78 ^{B,a}
3	5.00±0.01 ^{A,a}	4.73±0.22 ^{A,a}	4.44±0.34 ^{B,b}	3.88±0.23 ^{C,b}
4	4.76±0.15 ^{A,b}	4.00±0.06 ^{B,b}	3.89±0.09 ^{B,cd}	3.23±0.02 ^{B,c}
5	5.00±0.01 ^{A,a}	4.06±0.06 ^{B,b}	3.80±0.09 ^{B,cd}	3.62±0.02 ^{B,c}
6	5.00±0.01 ^{A,a}	4.00±0.06 ^{B,b}	3.71±0.09 ^{B,cd}	3.22±0.02 ^{C,c}
7	4.00±0.15 ^{A,b}	4.00±0.06 ^{B,b}	3.66±0.15 ^{B,d}	3.18±0.02 ^{C,c}
C	5.00±0.01 ^{A,a}	4.89±0.71 ^{B,a}	4.77±0.11 ^{B,a}	4.00±0.78 ^{C,a}
Overall acceptance scores				
1	5.00±0.09 ^{A,a}	4.60±0.58 ^{B,b}	4.50±0.00 ^{C,a}	3.80±0.19 ^{D,a}
2	5.00±0.09 ^{A,a}	4.42±0.00 ^{A,c}	3.17±0.10 ^{B,b}	3.75±0.02 ^{B,b}
3	5.00±0.09 ^{A,a}	4.37±0.00 ^{A,c}	3.10±0.10 ^{B,b}	3.61±0.02 ^{C,b}
4	5.76±0.90 ^{A,a}	3.22±0.00 ^{B,c}	3.00±0.00 ^{B,d}	3.23±0.11 ^{B,c}
5	5.00±0.09 ^{A,a}	3.13±0.06 ^{B,d}	3.09±0.07 ^{B,c}	3.17±0.18 ^{B,d}
6	5.00±0.09 ^{A,a}	4.13±0.00 ^{B,d}	3.09±0.06 ^{B,ac}	3.10±0.18 ^{C,d}
7	4.50±0.12 ^{A,b}	4.00±0.00 ^{B,de}	3.00±0.06 ^{B,c}	3.10±0.18 ^{C,d}
C	5.00±0.09 ^{A,a}	4.70±0.00 ^{B,a}	4.53±0.00 ^{B,a}	3.89±0.19 ^{C,a}

^a (A-D) and (a-e): Data are expressed as mean±standard deviation (n= 3). Values within each type of treatment method marked by the same letter within 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.

treatment with 60 mg kg⁻¹ *S. officinalis* extract ($P > 0.05$) but indicated a significant difference from other treatments and control sample ($P < 0.05$) (Table 5). Over time, a significantly descending trend was observed in the consistency scores. However, the consistency scores of treatment with *C. copticum* extract were higher than the treatment with *S. officinalis* extract. On the final day, the highest score was found for treatment with 20 mg kg⁻¹ *C. copticum* extract, which showed a significant difference from all treatments ($P < 0.05$), but not the control sample ($P > 0.05$). The lowest consistency score was related to treatment with a mixture of both extracts, indicating no significant difference from treatment with different concentrations of *S. officinalis* extract ($P > 0.05$) but showing a significant difference from other treatments and the control sample ($P < 0.05$) (Table 5).

In conclusion, it can be said that *S. officinalis* and *C. copticum* extracts had greater effects on the final quality of sausage samples in terms of physio-chemical and microbial properties and sensory attributes, respectively. However, these findings can be important due to the presence of different concentrations of *S. officinalis* and *C. copticum* extracts in the formulation of a product with less nitrite. The overall acceptance scores of treatments with *C. copticum* extract were higher than those of treatments with *S. officinalis* extract and lower than those of the control sample. On the final day, the highest score among the treatments were reported for 10 mg kg⁻¹ *C. copticum* extract, which showed a significant difference from other treatments ($P < 0.05$), but not from the control sample ($P > 0.05$) (Table 5).

DISCUSSION

According to Davies and Board (1998), the changes of pH value during the refrigerated storage of meat products is mainly due to the production of materials such as ammonia derived from the microorganisms activity,

proteins denaturation, or accumulation of the metabolite such as citric acid, H₂ and CO₂. In this study, the decrease in pH value of the treatments was due to the acidic nature of the *C. copticum* or *S. officinalis* extracts (Table 2). The effect of pH can be attributed either to the direct effect of pH or to the better dissolving of the extract in the lipid phase of the bacterial membrane at the lower pH= 3.7. Lowering the pH of a food product or beverage has been used as a preserving method (Theron and Lues, 2011).

Cooking loss in sausage depends on the extracts content or hydrocolloids used in the formulation. Use of plant extracts have either no effect on the cooking loss or have a reducing effect. In contrast, hydrocolloids decrease the cooking loss due to inhibitory effects against moisture discharge during the frying process, which is due to their ability in creating hydrogen connections with water molecules, which in turn prevents the humidity discharge during the frying process (Kamani *et al.*, 2019). The results showed that the cooking loss values gradually increased with an increase in the storage time from day 1 to day 45, showing the peak in the last month. With an increase in the shelf life from day 1 to 45, the cooking loss level gradually increased, which was more intense during the last month. The mean cooking loss was higher in treatment with the mixture of both extracts than those of the single extracts and the control sample. It can be argued that a critical concentration is sufficient to achieve a desirable antioxidant activity, following which the saturation effect appears. In line with this result, Fernandez-Lopez *et al.* (2005) reported that increased concentration of *Thymus vulgaris* extract had peroxidant effects on the product. This reduction and elevation in peroxide value, which has no specific trend, is possibly due to decomposition of hydroxides into carbonyl compounds, i.e. faster decomposition rather than formation of hydroperoxide (Georgantelis *et al.*, 2007).

Hydroperoxides are the primary products of oxidation of unsaturated fatty acids. Flavonoids are able to inhibit hydroxyl



radicals, superoxide, and lipid peroxyl radicals. The antioxidant effects of the compounds in medicinal plants are dependent upon the phenolic compounds or conjugated circular structures and hydroxyl groups that neutralize the free radicals as well as carboxylic acid compounds that prevent oxidation by chelating the metals (Kurutas, 2016). According to Table 2, the peroxide value increased as an increase occurred in the shelf life. In agreement with the present study, (Heydarian *et al.*, 2016) reported rosemary extract could delay lipid oxidation in the chicken fillets. Further, (Pirooti *et al.*, 2014) showed that use of *T. vulgaris* reduced the peroxide value in the sausages, which is in agreement with the results of this study, especially in treatment with a single administration of both extracts. Viuda- (Martos *et al.* 2009) investigated the effect of *Origanum vulgare* essence and thymus extract on bologna sausage and showed that these compounds increased the oxidative stability.

It has been reported that the antimicrobial activity of *S. officinalis* essence and extract can be due to high flavonoids content (Sharififar *et al.*, 2007). Since thymol and para-cymene are the main components of *C. copticum* essence, the antimicrobial activity of the extract and essence can be attributed to these compounds (Aberoomand *et al.*, 2010). There is a symbiosis between carvacrol and its precursor para-cymene, which is highly important as para-cymene swells up the cell membrane of microorganisms, facilitates the entry of higher carvacrol content into the cells, and, consequently, destroys the microorganisms. The antioxidant properties of the hydroxyl group in phenolic compounds creates a bond with the active enzymes and prevents their metabolism (Neito, *et al.* 2010). The antibacterial activities of *S. officinalis* extract in the food systems and the environment against *E. coli*, *S. aureus* and *Streptococcus* species are due to the presence of its bioactive compound, which is comparable to gentamicin, tetracycline, and chloramphenicol. The results in Table 4,

show that the use of these extract, specially *S. officinalis*, can be an appropriate relative alternative to nitrite as a synthetic additive compared to the control sample. The coliform contamination of meat and meat products can be attributed to the personnel, raw materials, factory environment, and equipment during the production and packaging processes (Sachindra *et al.*, 2005). In this regard, (Mengsha *et al.*, 2014) investigated the combined use of rosemary and nisin extracts to increase the shelf life of fish during storage, and reported significant reduction in the growth of coliforms and increased shelf life of each of the extracts used alone and in combination.

The presence of phenolic, terpenoid, and alkaloid compounds has been considered highly important in the incidence of antifungal activities of the plant extracts. The antimicrobial activity of *S. officinalis* extract may be due to the balanced presence of oxygenated carvacrol, thymol, terpinen-4-ol, and alpha-terpineol, which show a wide range of antifungal activities (Ksouri *et al.*, 2017). It is noteworthy that the antimicrobial effects of *S. officinalis* and *C. copticum* extracts are definitely higher in the culture medium than in a food with complex formulation like sausage, because the ingredients of the food decrease the activities of the extract through both creating a protective layer for the microorganism and combining with the active ingredients of the extracts. According to Table 5, the lowest score on the final day was related to the treatment with a mixture of both extracts. Kant *et al.* (2008) observed that the sausage samples containing mint extract were not significantly different from the control samples in terms of flavor. (Khaleghi *et al.* 2012) showed that use of *Berberis vulgaris* extract in the formulation of sausage indicated the highest score with regard to flavor.

(Viuda-Martos *et al.* 2010) reported that adding citrus wastes, 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 increasing 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. Due to the absence of proteins and carbohydrates in plant extracts, these ingredients will not have the same effect on consistency as on the color and flavor of the sausage, so they will not have the same effect on consistency as on the color and flavor of the sausage.

The results of this study showed that certain concentrations of *C. copticum* and *S. officinalis* extracts should be selected to achieve the optimal qualitative properties of sausage. As a conclusion, the results of cooking loss values and peroxide values during storage time showed that the extracts improved the qualitative properties of sausages. This is a clear evident that the extracts are effective agents in increasing shelf life and microbiological analysis and sensory evaluations exhibited supportive results for increasing shelf life. Also, the studied herbal extracts improved sensory properties of sausages. Treatment containing 40 mg kg⁻¹ *S. officinalis* extract was selected as a better treatment due to physicochemical, microbial and sensorial characteristics during refrigerated storage. The results of this study showed that by using the extracts of medicinal plants, synthetic additives such as nitrite and various salts in the formulation of meat products can be greatly reduced.

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تأثیر عصاره گیاه *Carum copticum* L. (زنیان) و *Salvia officinalis* L. (مریم گلی) بر خصوصیات فیزیکی شیمیایی، میکروبی و خصوصیات حسی سوسیس حرارت دیده در طول نگهداری یخچالی

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چکیده

در پژوهش حاضر از ۱۰، ۲۰ و ۳۰ میلی گرم بر کیلوگرم عصاره زنیان، ۴۰، ۵۰ و ۶۰ (mg/kg) عصاره مریم گلی و ترکیبی از این عصاره‌ها (۳۰ mg/kg: ۱۵) به عنوان جایگزین نسبی نیتريت در فرمولاسیون سوسیس استفاده شدند. سپس آزمایشات فیزیکی - شیمیایی، میکروبی و حسی بر روی کلیه تیمارها و نمونه شاهد (بدون عصاره) طی ۴۵ روز انجام شد. نتایج نشان داد که تیمار حاوی ۶۰ mg/kg عصاره مریم گلی کمترین مقدار pH را داشت. کمترین عدد پراکسید مربوط به تیمار با ۴۰ mg/kg در کیلوگرم عصاره مریم گلی بود که تفاوت معنی داری با نمونه شاهد نشان داد. نمونه شاهد بالاترین شمارش میکروبی را نشان داد. تیمار حاوی ۶۰ mg/kg عصاره مریم گلی کمترین میانگین تعداد کلستریدیوم پرفرانژنز را نشان داد. کمترین تعداد کلیفرم و کپک و مخمر برای تیمار ۶۰ mg/kg عصاره مریم گلی یافت شد. از نظر خصوصیات حسی، تیمار ۳۰ mg/kg عصاره زنیان بالاترین امتیاز رنگ و طعم را داشت، در حالی که بالاترین امتیاز قوام و پذیرش کلی به ترتیب مربوط به تیمار حاوی ۲۰ و ۱۰ mg/kg عصاره زنیان بود. نتایج این مطالعه نشان داد که برای دستیابی به خصوصیات کیفی مطلوب سوسیس باید غلظت مشخصی از دو عصاره انتخاب شود. به طور کلی، تیمار با غلظت‌های مختلف عصاره مریم گلی پذیرش بهتر و تأثیر بیشتری بر کیفیت نمونه‌های سوسیس داشت. به عنوان نتیجه‌گیری، تیمار حاوی ۴۰ mg/kg عصاره مریم گلی به دلیل خصوصیات فیزیکی - شیمیایی، میکروبی و حسی طی زمان نگهداری به عنوان تیمار بهتر انتخاب شد.