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Caraway (*Carum carvi* L.) essential oil improves quality of dry-fermented sausages produced with different levels of sodium nitrite

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Abstract

Caraway (*Carum carvi* L.) essential oil (CEO: 0.01, 0.05, 0.10 μl/g) was used in dry-fermented sausages manufactured with 15% and 25% of fat and three levels of sodium nitrite (0, 75, 150 mg/kg). Dry-fermented sausages were vacuum packed and stored (225 days) and their physico–chemical, microbial, and sensory attributes were assessed (a total of 288 dry-fermented sausages were produced). The inclusion of CEO had a significant effect on pH, texture and color, odor, and flavor. Moreover, the partial and total replacement of sodium nitrite with CEO (0.01 μl/g) provided acceptable TBARS values (<0.3 mg MDA/kg) in low-fat (15%) of sausages throughout the storage. The usage of CEO at 0.10 μl/g affected formation of atypical flavor. Generally, our findings confirmed that the CEO has promising potential for development of dry-fermented sausages with reduced level of sodium nitrite, primarily regarding their oxidative stability.

Practical applications

The usage of natural antioxidants is one of the key strategies in the novel meat industry. Concerning strong antioxidant potential, CEO could be used as an emerging antioxidant and potential substitute for sodium nitrite in meat processing.

1 | INTRODUCTION

Dry-fermented sausages are one the most popular dry-cured meat products (Tomović et al., 2020). The production of this meat product type is characterized by using the comminuted raw meat materials, high content of animal fat and absence of thermal treatments; hence these products are predisposed to spoilage, dominantly by lipid oxidation and bacterial growth (Šojić et al., 2014). The use of synthetic and/or natural additives is recommended in meat industry in order to obtain the safety of final products during long storage period (Tomović et al., 2020).

Sodium nitrite is mostly used as synthetic additive against spoilage and pathogenic bacteria in dry-fermented sausages (Ozaki et al., 2020; Šojić et al., 2020; Tomović et al., 2020). Moreover, sodium nitrite shows a strong potential in control of lipid oxidation (Tang et al., 2021) and emerging reddish-pink color and typical flavor for cured meat products (Perea-Sanz et al., 2020). However, nitrites can lead to the *N-nitrosamines* development with strong toxic, teratogenic, and carcinogenic potential (Alirezalu et al., 2021; Perea-Sanz et al., 2020; Tang et al., 2021). Consequently, one of the key challenges in the novel meat technology is finding alternatives for sodium nitrite (Alirezalu et al., 2021; Kurćubić et al., 2014; De Oliveira et al., 2011; Ozaki et al., 2020; Perea-Sanz et al., 2020). Due to wide spectrum of bioactive compounds (e.g., terpenoids and polyphenols) with proven health potential (Chen et al., 2021; Stojanović-Radić et al., 2018; Vasiljević et al., 2017), essential oil isolated from different plant origins (aromatic and medicinal) are progressively utilized as alternatives for synthetic additives, including sodium nitrite (Šojić et al., 2019; Tomović et al., 2020). Also, the increasing interest for natural additives could be related to some weaknesses of synthetic additives in relations of human toxicity and undesirable ecological impacts (Chen et al., 2021).

Caraway (*Carum carvi* L.) of the Apiaceae family is one of the ancient aromatic and medicinal plants all over the world (Rasooli & Allameh, 2016). The caraway seeds contain a relatively high percentage of essential oils (>40%) with significant nutraceutical and antioxidant potential (Hromiš et al., 2015; Krkić et al., 2013; Rasooli & Allameh, 2016). Hence, caraway essential oil (CEO) could serve as an effective food supplements and safe antioxidant and antimicrobial agent in prevention of food deterioration (Rasooli & Allameh, 2016). Furthermore, several studies assessed the effect of caraway essential on the shelf-life of dry-fermented sausages (Hromiš et al., 2015; Kocić-Tanackov et al., 2020; Krkić et al., 2013). Krkić et al. (2013) reported that CEO in combination with chitosan biopolymer efficiently improved oxidative state of *Petrovská klobása* sausage. In other studies, strong antibacterial (Hromiš et al., 2015) and antifungal (Kocić-Tanackov et al., 2020) potentials of CEO in preservation of dry-fermented sausages were confirmed.

Based on the literature data, we hypothesized that CEO oil can serve as alternative for sodium nitrite in processing of dry-fermented sausages. Thus, we assessed the supplementation of CEO (0.00, 0.01, 0.05, and 0.10 μl/g) on the pH, color, oxidative state, microbial quality, texture and sensory attributes (color, odor, flavor) of dry-fermented sausages manufactured with 15 and 25% of fat and three levels of sodium nitrite (0, 75, and 150 mg/kg).

2 | MATERIALS AND METHODS

2.1 | Caraway essential oil

The CEO, obtained by the steam distillation method, was purchased from the Herba doo producer (Belgrade, Serbia). CEO was retained in dark glass flasks at 4°C awaiting investigates.

2.1.1 | GC-MS profile of CEO

GC-MS profile of CEO (expressed as relative percentage) was determined by the method described in Pavlić et al. (2018), using gas chromatography system (Agilent GC890N) coupled to a mass spectrometer (Agilent MS 5759).

2.2 | Preparation of dry-fermented sausages

Dry-fermented sausages were produced according to the procedure described in our previous study (Tomović et al., 2020). Firstly, two batters were formulated with 15% and 25% of pork backfat. Sodium nitrite was added in both: 0, 75, and 150 mg/kg. A total of six sub-batters were produced. In each of them, CEO was added as a natural antioxidant at four concentrations: 0.00, 0.01, 0.05, and 0.10 μl/g. Furthermore, all of 24 batches (6 × 4) were subjected to following processes: stuffing (collagen casings − 37 mm diameter), smoking and ripening (t = 14–16°C; RH = 95%–80%) during 21 days. Finally, the obtained sausages were vacuum packed and stored at 15 ± 1°C for up to 225 days. Sausages were sampled at different times of storage (0, 75, 150, 225 days), including three randomly selected samples from each batch. A total of 288 dry-fermented sausages were produced.

2.3 | Physico–chemical analysis

The pH was measured using a Testo 205 digital pH meter (Testo AG, USA) equipped with a combined penetration tip.

The instrumental parameters of color (12 readings per sample) expressed as lightness—L^*, redness—a^*, and yellowness—b^* were measured using a MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan; instrumental details: D-65 lighting, a 2° standard observer angle and an 8-mm aperture in the measuring head; calibration plate: No. 11333090, Y = 92.9, x = 0.3159, y = 0.3322) in accordance with procedure described in Šojić et al. (2019).

Texture profile analysis (hardness, springiness, cohesiveness, and chewiness) of the samples was determined in accordance with procedure described in Tomović et al. (2020).

2-Thiobarbituric acid reactive substances test (TBARS), expressed as milligram of malondialdehyde (MDA) per kg of sample, was used to evaluate the degree of lipid oxidation (Šojić et al., 2019). All physico–chemical analysis were evaluated for three samples, from each group of sausages in duplicate.

2.4 | Microbiological analysis

Total aerobic mesophilic bacteria (TAMB), lactic acid bacteria (LAB), sulfite-reducing clostridia, *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* were determined on three samples from each group of dry-fermented sausages in duplicate, using standard ISO procedures: ISO 4833-1:2013, ISO 15214:1998, ISO 15213:2003, ISO 6579-1:2017, ISO 16649-2:2001, ISO 11290-2:2017, respectively.

2.5 | Sensory analysis

Prior to analyses panelists (10 members) were skilled in accordance with procedures described in ISO 8586:2015, ISO 8589:2007.

Difference-from-control test was used to evaluate the key sensory attributes of samples—color, odor, and flavor (Tomović et al., 2020). Prior to analyses, sausages were equilibrated to room temperature for about 15 min and marked with a three-digit sample number. The sausages were sliced into 2 mm thick pieces and placed on a white porcelain plate. Consumers were firstly questioned to evaluate the control sample (without CEO and with the corresponding contents of fat and nitrite) and afterward to determine how different the coded samples were from the control one. The difference was rated on a scale from 0 to 6, where 0 = no difference; 1 = very slight difference; 2 = slight/moderate difference; 3 = moderate difference; 4 = moderate/large difference; 5 = large difference; and 6 = very large difference.

2.6 | Statistical analysis

The main effects (content of fat, nitrite, and CEO content and day of storage) for all analyzed physico–chemical, microbiological, and sensory parameters were compared using *t*-test and Duncan's multiple range test ($p < .05$). All data were expressed as mean value with their standard deviations (*SD*). The two-way, three-way and four-way interactions between these effects were also tested.

3 | RESULTS AND DISCUSSION

3.1 | Chemical profile of CEO

CEO was characterized in terms of chemical profile of volatile terpenoid compounds prior to its addition in meat products (Table 1 and Figure 1). Carvone (77.00%) was recognized as the predominant compounds in CEO and its content was followed by D,L-limonene (18.05%). Mahboubi (2021) also reported that carvone and limonene accounted for 95% of CEO compounds (Mahboubi, 2021). Content of two major terpenoids was followed by dihydrocarvone (1.05%), while all other detected volatiles were observed in <1% relative percentage (Table 1). Oxygenated monoterpenes and monoterpenes hydrocarbons were identified as the leading subgroups of terpenoids due to high content of carvone and limonene, respectively (Figure 1) what was in align with literature data (Laribi et al., 2013). Sesquiterpenes (β-bourbonene, caryophyllene, trans-beta-farnesene, and caryophyllene oxide) were the minor compounds presented in these sample, while certain portion of unidentified compounds could be attributed to the esters, aldehydes, sesquiterpene hydrocarbons, etc. (Laribi et al., 2013). Climate conditions, time of harvest, water content, geographical conditions, genotype, and postharvest processing often exhibit significant influence on yield and chemical profile of essential oil, however, only few of them will alter the content of carvone and limonene, while changes in minor essential oil compounds could be significant (Mahboubi, 2021).

It was reported that caraway seeds exhibit antioxidant activity in various in vitro model systems including reducing power assays, DPPH, and β-carotene–linoleic acid (Rasooli & Allameh, 2016). Furthermore, Fatemi et al. (2010) informed that antioxidant activity of CEO was significantly higher comparing to trolox in DPPH and β-carotene bleaching assay. On the other hand, antibacterial activity of CEO was confirmed on the common food-borne pathogens such as *Bacillus cereus*, *L. monocytogenes*, *Salmonella enteritidis*, and *E. coli* O157:H7 (Oroojalian et al., 2010). Clearly stated antioxidant and antimicrobial properties of CEO were the major motivation for selecting this sample as natural functional additive in dry-fermented sausages.

TABLE 1 Chemical profile of CEO sample

Compound	RT[1] (min)	Relative percentage (%)
Sabinene	4.35	0.02
n.i.[2]	4.43	0.01
n.i.	4.59	0.02
Beta-Myrcene	4.68	0.06
p-Cymene	5.49	0.03
D,L-Limonene	5.64	18.05
Linalool	7.48	0.06
n.i.	8.08	0.22
n.i.	8.21	0.20
n.i.	8.35	0.02
cis-Limonene-oxide	8.40	0.03
n.i.	8.51	0.20
n.i.	9.19	0.04
n.i.	9.33	0.05
n.i.	9.77	0.06
n.i.	10.23	0.30
Dihydrocarvone	10.37	1.05
n.i.	10.59	0.55
Isodihydrocarvenol	11.20	0.38
Trans-Carveol	11.43	0.55
Carvone	12.22	77.00
n.i.	12.82	0.25
n.i.	12.96	0.12
n.i.	14.31	0.08
n.i.	15.00	0.18
n.i.	15.99	0.08
Beta-Bourbonene	16.19	0.07
n.i.	16.97	0.04
n.i.	17.06	0.04
Caryophillene	17.24	0.07
Trans-beta-Farnesene	18.42	0.03
Caryophyllene oxide	22.10	0.17
Total		100

[1]Retention time (min).
[2]Not identified.

3.2 | Physico–chemical parameters of dry-fermented sausages

Physico–chemical parameters (pH, color—*CIEL*a*b**, and TBARS values) are displayed in Table 2. Backfat had a significant ($p < .05$) effect on the pH value. The increase of fat accelerated the process of lipolysis and formation of free fatty acids, what consequently affected decrease of pH value (Tomović et al., 2020). Also, sodium nitrite, CEO and storage time had significant ($p < .05$) effects on the pH value. Generally, increase of pH value was followed by proteolysis and decline of LAB (Kurćubić et al., 2014). Two-way (BF ×CEO), three-way (BF × SN × ST; BF × SN × CEO; BF × ST × CEO), and four-way interaction (BF × SN × ST × CEO) also had significant effects on the pH value ($p < .05$–.001; Table S1—Supporting Information).

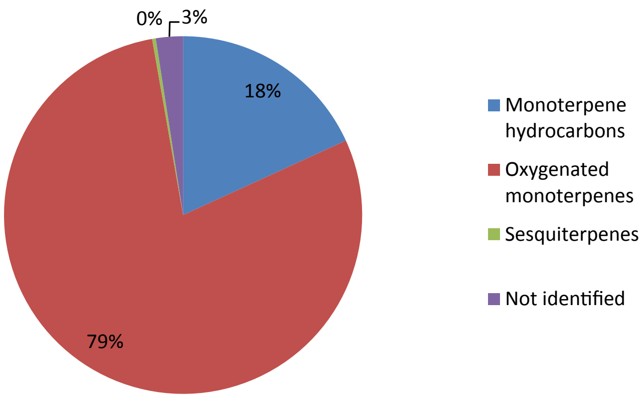

FIGURE 1 Relative percentage of terpenoid subgroups observed in CEO

Color is still the most important meat quality attribute perceived by consumers (Tomasevic et al., 2021). In the case of color, backfat significantly ($p < .05$) increased the lightness and decreased the redness and yellowness. The color results are consistent with those reported by Tomović et al. (2020) and Šojić et al. (2021). Moreover, sodium nitrite had a significant ($p < .05$) effect in declining the lightness and increasing the yellowness. This phenomenon could be related to nitrosyl myoglobin formation (Ozaki et al., 2021). The usage of natural antioxidant (CEO) significantly ($p < .05$) decreased the lightness and increased the yellowness. Probably, it was the result of interactions among terpenoids of CEO (e.g., carvone, limonene) and pigment myoglobin (Jin et al., 2018; Tomović et al., 2020). Storage time significantly ($p < .05$) decreased the lightness, but increased the redness and yellowness. Findings are consistent with those reported in our previous study (Šojić et al., 2021). The two-way interactions (BF × SN and BF × ST) had significant effects ($p < .05$) on all three parameters of color (Table S1). In the case of four-way interaction, it can be observed that BF × SN × ST × CEO had a significant ($p < .05$) effect on the redness and the yellowness. The individual values of redness ranged in interval from 9.35 (BF = 25%; SN = 0 mg/kg; CEO = 0.00 μl/g; ST = 0) to 16.83 (BF = 15%; SN = 75 mg/kg; CEO = 0.10 μl/g; ST = 225). The obtained results confirmed synergistic effect of sodium nitrite (75 mg/kg) and CEO (0.10 μl/g) in increased redness of low-fat dry-fermented sausages. Similarly, in our previous studies we determined that essential oil obtained from juniper (Tomović et al., 2020) and sage (Šojić et al., 2021) efficiently improved the redness of dry-fermented sausages produced with different levels of sodium nitrite. Beside traditional

TABLE 2 pH, instrumental parameters of color and TBARS values of dry-fermented sausages

	pH	*CIEL**	*CIEa**	*CIEb**	TBARS (mg MDA/kg)
Backfat content (%)					
15	5.47 ± 0.07^{a}	47.7 ± 3.0^{b}	14.0 ± 1.7^{a}	8.09 ± 1.30^{a}	0.14 ± 0.09^{a}
25	5.35 ± 0.08^{b}	52.4 ± 3.4^{a}	12.8 ± 1.7^{a}	7.60 ± 1.13^{a}	0.15 ± 0.10^{a}
Sodium nitrite content (mg/kg)					
0	5.38 ± 0.10^{b}	50.9 ± 4.2^{a}	13.2 ± 2.1^{a}	7.60 ± 1.31^{b}	0.18 ± 0.11^{a}
75	5.43 ± 0.10^{a}	50.0 ± 4.1^{b}	13.5 ± 1.8^{a}	7.87 ± 1.23^{a}	0.12 ± 0.09^{b}
150	5.42 ± 0.09^{a}	49.3 ± 3.5^{b}	13.5 ± 1.5^{a}	8.07 ± 1.15^{a}	0.13 ± 0.08^{b}
CEO content (μl/g)					
0	5.37 ± 0.10^{c}	51.2 ± 4.2^{a}	13.3 ± 1.9^{a}	7.80 ± 1.13^{a}	0.20 ± 0.11^{a}
0.01	5.45 ± 0.09^{a}	49.4 ± 3.8^{b}	13.2 ± 1.8^{a}	7.73 ± 1.26^{a}	0.14 ± 0.10^{b}
0.05	5.40 ± 0.10^{b}	50.3 ± 3.8^{b}	13.5 ± 1.7^{a}	7.97 ± 1.24^{a}	0.13 ± 0.09^{bc}
0.10	5.42 ± 0.08^{ab}	49.5 ± 3.9^{b}	13.6 ± 1.9^{a}	7.89 ± 1.32^{a}	0.10 ± 0.07^{c}
Storage time (day)					
0	5.35 ± 0.08^{d}	51.2 ± 4.0^{a}	12.6 ± 2.0^{c}	7.25 ± 1.17^{c}	0.03 ± 0.02^{c}
75	5.47 ± 0.09^{a}	50.4 ± 4.1^{ab}	13.3 ± 1.5^{b}	7.77 ± 0.92^{b}	0.12 ± 0.07^{b}
150	5.39 ± 0.08^{c}	49.6 ± 3.8^{bc}	13.4 ± 1.8^{b}	7.75 ± 1.24^{b}	0.20 ± 0.05^{a}
225	5.43 ± 0.10^{b}	49.1 ± 3.8^{c}	14.3 ± 1.6^{a}	8.61 ± 1.21^{a}	0.22 ± 0.10^{a}

colorimeter (Minolta), color values of the meat products could be measured using computer vision system. According to Tomasevic et al. (2019) computer vision system should be considered a more desirable alternative to the traditional method for measuring color of dry-fermented sausages.

It is well known that oxidation of lipids is one of the main chemical deterioration that limits the shelf life of different type of meat products (Ozaki et al., 2021). Melton (1983) recommended that TBARS value of 0.3 mg MDA/kg could be marked as the threshold for rancidity of meat products. The sodium nitrite and CEO had significant ($p < .05$) effects on the TBARS values (Table 2). Ozaki et al. (2021) reported that reactions between sodium nitrite and potential prooxidants (heminic and nonheminic iron) delay oxidative reactions in meat products. In the case of natural antioxidant, it is important to highlight a strong antioxidant activity of CEO in dry-fermented sausages. According to Samojlik et al. (2010), antioxidant effects of CEO determined by in vitro and in vivo tests can be related to its predominant compound, carvone. Furthermore, it was indicated in the same study that CEO could serve as pharmaceuticals, flavor agent, as well as natural additive in wide spectrum of foodstuffs and beverages (Samojlik et al., 2010).

Finally, TBARS values significantly ($p < .05$) increased throughout the storage, undoubtedly as the result of lipid oxidative changes in similar meat product (Krkić et al., 2013). The following two-way (BF × SN; BF × ST; SN × ST; ST × CEO), three-way (BF × SN × ST) and four-way (BF × SN × ST × CEO) interactions had significant ($p < .05$–.001) effects on TBARS values (Table S1). Also, it is important to highlight that in following samples: BF = 15%, SN = 75 mg/kg, CEO = 0.00 μl/g, ST = 225; BF = 15%, SN = 0 mg/kg, CEO = 0.00 μl/g, ST = 225; BF = 25%, SN = 0 mg/kg, CEO = 0.00 μl/g, ST = 225; BF = 25%, SN = 0 mg/kg, CEO = 0.01 μl/g, ST = 225, and BF = 25%, SN = 0 mg/kg, CEO = 0.05 μl/g, ST = 225, TBARS values were higher than 0.3 mg MDA/kg. Hence, 0.01 μl/g of CEO improved oxidative shelf-life (225 days) of no nitrite sausages.

3.3 | Microbiological profile of dry-fermented sausages

TAMB and LAB counts are presented in Table 3. The backfat, sodium nitrite, and CEO did not significantly ($p > .05$) effect on the TAMB and LAB counts. Findings are consistent with those reported in our previous studies (Šojić et al., 2021; Tomović et al., 2020). In the case of antimicrobial activity of essential oils, it is important to highlight that further investigations in terms of plant materials selection, extraction techniques (e.g., hydrodistillation, ultrasound extraction), as well as optimization of their concentrations is necessary. Even though hydrodistillation is regarded as traditional approach in EO isolation, this technique is still considered as method of choice since it could provide selective separation of volatile fraction particularly concentrated with terpenoids. Seidler-Łożykowska et al. (2013) reported that antimicrobial effects of CEO strongly depend on the content of its minor components, rather than carvone.

TABLE 3 Microbiological quality of dry-fermented sausages

	Aerobic plate count (log CFU/g)	LAB (log CFU/g)
Backfat content (%)		
15	5.52 ± 0.83^a	5.60 ± 0.84^a
25	5.42 ± 0.87^a	5.51 ± 0.75^a
Sodium nitrite content (mg/kg)		
0	5.46 ± 0.84^a	5.66 ± 0.76^a
75	5.47 ± 0.81^a	5.59 ± 0.74^a
150	5.48 ± 0.89^a	5.41 ± 0.86^a
CEO content (μl/g)		
0	5.51 ± 0.96^a	5.62 ± 0.70^a
0.01	5.37 ± 0.80^a	5.49 ± 0.89^a
0.05	5.53 ± 0.79^a	5.57 ± 0.90^a
0.10	5.47 ± 0.85^a	5.53 ± 0.82^a
Storage time (day)		
0	5.00 ± 0.38^b	6.51 ± 0.25^a
75	4.51 ± 0.32^c	5.46 ± 0.50^b
150	6.30 ± 0.36^a	5.58 ± 0.42^b
225	6.07 ± 0.56^a	4.66 ± 0.57^c

Storage time had a significant ($p < .05$) effect on the microbiological profile of dry-fermented sausages. TAMB inconsistently increased throughout the storage (4.51–6.07 log CFU/g). These results are consistent with those reported in our previous study (Tomović et al., 2020). LAB counts decreased during the storage time (Table 3). Ozaki et al. (2021) observed that absence of sugar and low storage temperature are responsible for reduction of LAB. Also, none of interactions (two-way, three way, and four-way) did not show significant ($p > .05$) effect on the microbiological profile of dry-fermented sausages (Table S1). Furthermore, the most abundant pathogen bacteria in cured meat products (sulfite-reducing clostridia, *Salmonella* spp., *E. coli*, and *L. monocytogenes*) were not found in any samples. The absence of pathogen bacteria and relatively low TAMB counts (Table 3) may be the result of high-quality raw materials and good hygienic procedures during meat processing (Perea-Sanz et al., 2020).

3.4 | Texture profile of dry-fermented sausages

Texture attributes (hardness, springiness, cohesiveness, and chewiness) are presented in Table 4. Backfat had a significant ($p < .05$) effect on all texture attributes of dry-fermented sausages. Findings were in a strong agreement with those reported in our previous study (Šojić et al., 2021). In the case of antioxidants addition (sodium nitrite vs. CEO), it should be noticed that the CEO had higher impact on the texture attributes. The CEO addition affected ($p < .05$) increase of hardness and chewiness. These data are in contrary with those reported in our previous studies, where we showed that the same concentrations of juniper (Tomović et al., 2020) and sage

TABLE 4 Texture attributes of dry-fermented sausages

	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)
Backfat content (%)				
15	7,697 ± 1,371[a]	0.49 ± 0.04[a]	0.51 ± 0.03[b]	1,920 ± 412[a]
25	5,474 ± 990[b]	0.51 ± 0.05[a]	0.53 ± 0.04[a]	1,487 ± 313[b]
Sodium nitrite content (mg/kg)				
0	6,480 ± 1,523[a]	0.50 ± 0.05[a]	0.51 ± 0.04[b]	1,647 ± 411[a]
75	6,583 ± 1,681[a]	0.51 ± 0.05[a]	0.52 ± 0.03[a]	1,718 ± 439[a]
150	6,686 ± 1,680[a]	0.50 ± 0.05[a]	0.52 ± 0.04[a]	1,745 ± 418[a]
CEO content (μl/g)				
0	6,269 ± 1,752[c]	0.51 ± 0.05[a]	0.52 ± 0.04[a]	1,654 ± 449[b]
0.01	6,719 ± 1,781[ab]	0.49 ± 0.05[b]	0.51 ± 0.04[ab]	1,667 ± 407[b]
0.05	6,343 ± 1,416[bc]	0.50 ± 0.05[ab]	0.52 ± 0.04[a]	1,672 ± 384[b]
0.10	6,998 ± 1,437[a]	0.50 ± 0.05[ab]	0.51 ± 0.03[b]	1,819 ± 434[a]
Storage time (day)				
0	5,294 ± 1,135[c]	0.46 ± 0.05[c]	0.54 ± 0.04[a]	1,315 ± 269[c]
75	6,661 ± 1,353[b]	0.51 ± 0.04[b]	0.53 ± 0.03[b]	1,762 ± 283[b]
150	7,498 ± 1,401[a]	0.51 ± 0.03[b]	0.51 ± 0.03[c]	1,928 ± 362[a]
225	6,873 ± 1,731[b]	0.53 ± 0.03[a]	0.50 ± 0.03[d]	1,806 ± 474[b]

essential oil (Šojić et al., 2021) had no effect on analyzed texture attributes. Decreased tenderness of meat products could be the result of protein oxidation and the creation of protein cross-links (Jongberg et al., 2013), despite the addition of essential oils whose main goal was to inhibit both lipid and protein oxidative deterioration. The ability of the CEO to inhibit lipid oxidation was evident (from the Table 2), but the protein oxidation and products formations are more complex (Jongberg et al., 2013). Pateiro et al. (2015) reported significant differences in hardness values with antioxidants addition. Samples with the lowest concentration of CEO had the highest value for hardness, followed by the samples with the highest added concentration of antioxidant. The medium concentration of added antioxidant had the lowest values of hardness, suggesting the lower protein oxidation (Pateiro et al., 2015). In order to obtain optimal texture of dry-fermented sausages, the selection of natural antioxidants and optimization of their concentrations are essential. As expected, the storage time significantly ($p < .05$) affected the texture attributes. In accordance with our previous study (Šojić et al., 2021), hardness and chewiness significantly increased ($p < .05$) till 150th day of storage, then followed by decrease ($p < .05$) till day 225. The decrease of hardness and chewiness values could be the results of lipid oxidation (Šojić et al., 2021). The two-way (BF × ST) and four-way (BF × SN × ST × CEO) interactions also had significant ($p < .01$–$.001$) effects on all texture attributes (Table S1).

3.5 | Sensory quality of dry-fermented sausages

The following sensory attributes: color, odor, and flavor are presented in Table 5. Backfat, sodium nitrite, CEO, and storage time had significant ($p < .05$) effects on the color, but the differences among the samples were very slight (<1). Findings are consistent with those reported in our previous study (Tomović et al., 2020). Also, the following two-way (BF × SN; BF × CEO; SN × CEO), three-way (BF × SN × CEO; SN × ST × CEO), and four-way (BF × SN × ST × CEO) interactions had significant ($p < .001$–$.05$) effects on the color (Table S1).

TABLE 5 Sensory attributes of dry-fermented sausages

	Color	Odor	Flavor
Backfat content (%)			
15	0.25 ± 0.56[b]	0.50 ± 0.92[a]	0.82 ± 1.13[b]
25	0.80 ± 0.92[a]	0.55 ± 0.93[a]	0.91 ± 1.23[a]
Sodium nitrite content (mg/kg)			
0	0.60 ± 0.94[a]	0.48 ± 0.91[a]	0.86 ± 1.25[a]
75	0.54 ± 0.70[a]	0.54 ± 0.90[a]	0.87 ± 1.14[a]
150	0.44 ± 0.76[b]	0.56 ± 0.95[a]	0.85 ± 1.15[a]
CEO content (μl/g)			
0	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]
0.01	0.67 ± 0.83[b]	0.22 ± 0.54[c]	0.27 ± 0.60[c]
0.05	0.55 ± 0.73[c]	0.40 ± 0.66[b]	0.69 ± 0.79[b]
0.10	0.88 ± 0.99[a]	1.49 ± 1.16[a]	2.50 ± 0.90[a]
Storage time (day)			
0	0.41 ± 0.72[c]	0.66 ± 1.05[a]	1.01 ± 1.30[a]
75	0.52 ± 0.82[b]	0.50 ± 0.95[b]	0.71 ± 1.08[bc]
150	0.52 ± 0.69[b]	0.45 ± 0.76[b]	0.81 ± 1.12[c]
225	0.65 ± 0.97[a]	0.50 ± 0.88[b]	0.92 ± 1.21[ab]

Furthermore, CEO, storage time, as well as their interaction (ST × CEO) had significant ($p < .05$) effects on the odor. However, it should be noticed that the differences among the samples were ranged in interval from very slight (<1) to slight (<2).

Finally, backfat, CEO, and storage time had significant ($p < .05$) effects on the flavor. As expected, CEO showed the most pronounced effect on this attribute. It is important to highlight that the addition of 0.10 μl/g of CEO provided atypical flavor for this type of dry-cured meat products (Table 5). Strong impact of CEO on the flavor of dry-fermented sausages was unquestionably associated with its chemical profile. It is well known that hydrodistillation provides "pure" essential oil with a high percentage of terpenoids compounds (Table 1 and Figure 1), which possess a strong flavor (Šojić et al., 2018). Also, the following two-way (SN × CEO; ST × CEO), three-way (BF × SN × CEO; BF × ST × CEO), and four-way (BF × SN × ST × CEO) interactions had significant ($p < .05$–.001) effects on the flavor.

4 | CONCLUSIONS

The most abundant compound of CEO was monoterpene carvone (77%). The higher content of backfat allowed lower values for the following physico–chemical attributes: pH, hardness, and chewiness. In the case of antioxidant, sodium nitrite, and CEO addition decreased the lightness and TBARS values. Also, it is important to highlight that CEO at 0.10 μl/g contributed to the formation of atypical flavor for dry-fermented sausages. Furthermore, the least concentration of CEO (0.01 μl/g) efficiently increased the redness and improved oxidative shelf-life (TBARS <0.3 mg MDA/kg) of low-fat (15%) dry-fermented sausages produced with reduced levels of sodium nitrite (0 and 75 mg/kg). Hence, the obtained results suggested that the CEO (0.01 μl/g) could be used as a safe antioxidant and an effective sodium nitrite replacement in dry-fermented sausages processing.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Branislav V Šojić: Conceptualization; Writing-original draft. **Vladimir M Tomović:** Conceptualization; Resources; Supervision. **Jovo Savanović:** Formal analysis; Methodology; Resources. **Sunčica Kocić-Tanackov:** Formal analysis; Investigation. **Branimir Pavlic:** Validation; Writing-original draft. **Marija R Jokanović:** Project administration; Writing-review & editing. **Vesna Djordjevic:** Funding acquisition; Resources. **Nenad Parunovic:** Funding acquisition; Methodology. **Aleksandra Martinović:** Project administration; Supervision. **Dragan Vujadinović:** Software; Visualization.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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