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Extending the Shelf Life of Fresh Camel Sausage by the Integration of *Cuminum cyminum* L. Essential Oil

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ABSTRACT

Fresh sausage has a short shelf life and is easily spoiled in refrigerated conditions. The present study aimed to assess the effects of *Cuminum cyminum* L. essential oil (CCEO; 0%, 0.05%, 0.1% and 0.2% v/v) on the shelf life extension of fresh camel sausage within storage at the temperature of 4ºC for 15 days. According to the microbiological findings, the integration of CCEO significantly retarded microbial growth in the sausage compared to the control group (P<0.05). Mesophilic bacteria count (MBC) reached the upper microbiological permissible limit (7 log CFU/g) on day five in the control samples, on day seven in the samples containing 0.05% and 0.1% CCEO, and on day 15 in the samples containing 0.2% CCEO. According to the chemical findings, the total volatile base nitrogen (TVB-N) value in the control samples increased to 39.75 mg/100 g on the last day of storage. At the end of the research, a significant reduction (approximately 6.29-11.85 mg/100 g) was observed in the final TVB-N of the samples integrated with CCEO compared to the controls (P<0.05). The peroxide value (PV) of the control samples was 4.49 meq/1,000 g of lipids, while the PV values of the treated samples remained lower (3.25 meq/1,000 g of lipids) at the final stage of the study. In terms of sensory attributes, the addition of 0.05% and 0.1% CCEO caused slight adverse effects on the sensory characteristics of the samples (P>0.05). According to the results, the integration of CCEO with fresh camel sausages is a practical method to increase the shelf life of this product.

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Introduction

Camel meat is a valuable source of proteins, vitamins, and minerals for the inhabitants of arid and semi-arid areas. Typically, health-conscious consumers in Asian countries are interested in consuming this type of meat since it contains less fat and cholesterol content and relatively more polyunsaturated fatty acids compared to other types of red meat (1, 2). Moreover, camel meat is used as a treatment for diseases such as hypertension, shoulder pain, asthma, hyperacidity, seasonal fever, sciatica, and freckle removal and is known as an aphrodisiac as well (3-5).

Fresh sausages are among the oldest meat products, which are prepared from beef, buffalo, mutton, lamb, goat, camel, poultry, fish, and horse meat all over the world. These sausages are prone to lipid oxidation and microbial spoilage due to properties such as high water activity, high pH value, absence of chemical preservatives in their ingredients, and lack of thermal process in their production (6, 7).

Using herbal essential oils is a novel approach to the bio-preservation of fresh sausage (7). Essential oils are also referred to as ethereal or volatile oils, are aromatic oily complex mixtures of volatile compounds, which are commonly extracted from plants by the steam distillation technique. Essential oils are categorized as natural preservatives and have the potential to retard the growth of spoilage and pathogenic bacteria (7-9).

Cumin (*Cuminum cyminum* L.), with the vernacular name '*Zireh-e Sabz*' in Iran, is a small, herbaceous, annual-growing aromatic plant belonging to the Apiaceae family (10, 11). Geographically, *Cuminum cyminum* L. grows widely in Asia, North Africa, Europe, and the

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Americas and is also cultivated in the southeast of Asia and in Mediterranean countries (11, 12). For centuries, cumin seeds have been used as a flavoring agent in various foods and as a medication for dyspepsia, flatulence, diarrhea and parasitic diseases in traditional medicine (8, 12, 13).

The antibacterial, antiviral, antifungal, anti-insect, and antioxidant activities of *Cuminum cyminum* essential oil (CCEO) have been documented in several published studies (11-14). Given the scarce data regarding the microbial and chemical stability of fresh camel sausage during refrigerated storage, the present study aimed to assess the effects of various CCEO concentrations on the shelf life of camel sausage.

Materials and Methods

Essential Oil Extraction

Cuminum cyminum seeds were collected from Kerman, Iran in summer 2020, and their essential oil was extracted by mixing 100 grams of powdered *Cuminum cyminum* seeds with 1,500 milliliters of distilled water, and the mixture was heated using a Clevenger apparatus for three hours. The extracted essential oil was dehydrated using sodium sulfate and stored in an opaque glass at the temperature of 4°C for further assessment. The extracted essential oil was analyzed to identify the components using gas chromatography-mass spectrometry (GC-MS; Agilent 7890A/5975C, USA) in accordance with the protocol previously described by Talebi et al. (2108) (15).

Sausage Production

The semimembranosus muscle was segmented from camel carcass 24 hours after slaughtering in a local abattoir in Tehran province, Iran and delivered to the food hygiene laboratory in chilled condition within 30 minutes. Following that, the meat was ground using a sterile steel meat mincer from a plate with eight-millimeter orifices. Afterwards, salt, spices, and different concentrations of CCEO (0%, 0.05%, 0.1%, and 0.2%) were integrated with the minced meat and thoroughly mixed. In the next step, the mixtures were stuffed into lamb casings, transferred aseptically to bags, and stored at the temperature of 4°C for 15 days (7).

2.3. Microbiological Analysis

During the refrigerated storage period at six intervals (0, 3, 5, 7, 10, and 15 days), fresh camel sausage samples (10 g) were homogenized with 90 milliliters of sterile peptone water (0.1% w/v) for two minutes using a stomacher (Interscience, Bagmixer@400, France). Following that, different decimal dilutions were prepared by nine millimeters of diluent (0.1% sterile peptone water) and spread-plated on various agar media, including plate count agar, violet red bile glucose agar, *Pseudomonas* agar, and De Man Rogosa Sharpe agar (Quelab, Montreal, Québec, Canada). Bacterial enumeration was converted into logarithms of the number of colony-forming units (CFU/g) (7).

Chemical Analysis

Total volatile base nitrogen (TVBN; mg/100 g of sample) and the oxidative rancidity of the fresh sausage samples (peroxide value) were measured using the methods previously described by Sallam, Ishioroshi, and Samejima (2004) and Jouki et al. (2014), respectively (16, 17).

Sensory Analysis

The cooking of fresh camel sausage was done using an electric grill to the point that the core temperature reached 71°C. Following that, the center of the sausage samples was cut to rectangular pieces (2×1.5 cm; thickness: 1 cm). The samples were randomly coded to a three-digit blinded code and assessed by a nine-member trained panel at the same location with the same light and dish conditions. The panelists used palate cleansers unsalted crackers and room temperature distilled water in this process. The investigated characteristics of the samples included color, odor, and taste, which were rated from zero (least liked sample) to five (most liked sample) (7, 18).

2.6. Statistical Analysis

Data analysis was performed in SPSS version 16.0 for Windows using the analysis of variance (ANOVA), followed by Tukey's test. In all the statistical analyses, the significance level was set at $P<0.05$.

Results and Discussion

Chemical Composition of CCEO

The results of the GC-MS analysis of CCEO indicated the most dominant individual constituents to be 1,4-p-menthadien-7-al (32.20%) and cuminaldehyde (29.57%) in the extracted essential oil (Table 1). The amount of cuminaldehyde in CCEO was consistent with the previous studies in this regard, which indicated that cuminaldehyde is one of the major components of CCEO (11-13). The quality and quantity of herbal essential oils could be affected by the difference in the age and growth phase of the plant, the harvest season, geographic location, altitude, ground tilt, climate changes, soil conditions, and the method of extraction (7, 8, 19).

Table 1. Main components of *Cuminum cyminum* essential oil

No	RT(min)	Area%	Name	Quality
1	553.6	14.0	alpha-thujene	91
2	771.6	26.0	2-Pinene	97
3	042.8	20.0	Sabinene	96
4	224.8	16.5	beta-Pinene	97
5	748.8	31.0	beta-Myrcene	95
6	204.9	20.0	1-Phellandrene	97
7	915.9	46.5	Cymene	95
8	128.10	13.0	1,8-Cineole	92
9	581.11	11.10	gamma-Terpinene	97
10	153.13	02.0	delta,3-Carene	91
11	684.14	04.0	3-Methyl-2,4-hexadiene	76
12	349.16	19.0	4-Terpineol	96
13	738.16	88.0	3-Cyclopentylcyclopentan-1-one	83
14	935.16	02.0	Isoterpinolene	72
15	25.19	57.29	Cuminaldehyde	98
16	185.21	50.14	2-Caren-10-al	72
17	067.22	20.32	1,4-p-Menthadien-7-al	78
18	084.23	03.0	Myrtenal	76
19	249.26	03.0	Alloocimene	76
20	02.29	05.0	Trans-.beta.-Farnesene	55
21	466.29	13.0	UNKOWN FROM LIMEN OIL	93
22	255.30	03.0	cis-2-Methylenehexahydroindan-7-one	38
23	636.32	02.0	2-Methoxybenzyl alchohol	64
24	238.33	06.0	Carotol	90

Microbiological Analysis

Microbial growth is an influential factor in the shelf life of fresh sausages (20, 21). Figure 1 shows the mesophilic bacteria count (MBC) indicating the acceptable quality of four different batches of the fresh camel sausages on day zero in the present study. Accordingly, the initial MBC of the fresh camel sausage samples was 3.78 log CFU/g, which indicated that the products had acceptable microbiological quality. However, the estimated MBC was higher than other reports on camel minced meat (22). This discrepancy could be due to the microbiological level of untreated spices depending on the origin, method of harvesting, hygiene of transport, and storage of spices (21).

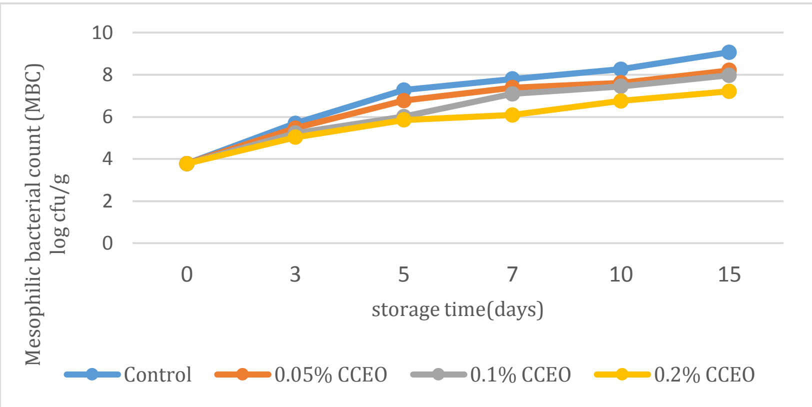

Figure 1. Changes in mesophilic bacterial count (MBC) of Fresh camel sausage during refrigerated storage (*Cuminum cyminum* essential oil (CCEO))

At the end of the current research, the MBC value reached 9.06 log CFU/ g in the control samples, while the lowest bacterial count was obtained in the sausage samples containing 0.2% CCEO (7.21

log CFU/g) (P<0.05). In the other treatments, the integration of the essential oil could significantly decrease or retard mesophilic bacterial growth in the sausages compared to the control samples (P<0.05). Furthermore, the MBC value reached the upper microbiological permissible limit (7 log CFU/g) on day five in the control samples, on day seven in the samples integrated with 0.05% or 0.1% CCEO, and on day 15 in the samples containing 0.2% CCEO. With regard to the use of CCEO in fresh camel sausage, our findings are in line with the study by Zhang et al. (2019), which indicated that the addition of 0.1% and 0.5% cinnamon essential oil to fresh pork sausage significantly decreased the MBC compared to the control samples (23). Similar findings have also been reported by Sojic et al. (2019), demonstrating that the MBC of fresh pork sausage incorporated with two concentrations of winter savory essential oil (0.075 and 0.150 µl/g) significantly decreased compared to control samples during the study period (23).

Our findings are consistent with the results obtained by Da Silveira et al. (2014), who reported that the integration of 0.1% bay leaf essential oil with fresh Tuscan sausage significantly decrease the MBC value compared to the controls, thereby resulting in a two-day extension of the shelf life of the treated samples stored at the temperature of 7°C (24). In another study, Ajourloo et al. (2020) reported that the MBC of fresh Balkan style sausage enriched with *Ziziphora clinopodioides* essential oil and lysozyme (alone or combined) significantly decreased compared to control samples (P<0.05)(7).

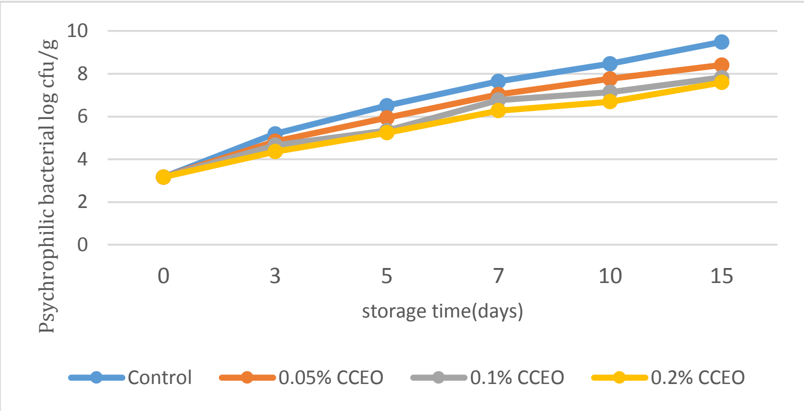

Figure 2. Changes in psychrotrophic bacteria (PSB) of Fresh camel sausage during refrigerated storage (*Cuminum cyminum* essential oil (CCEO))

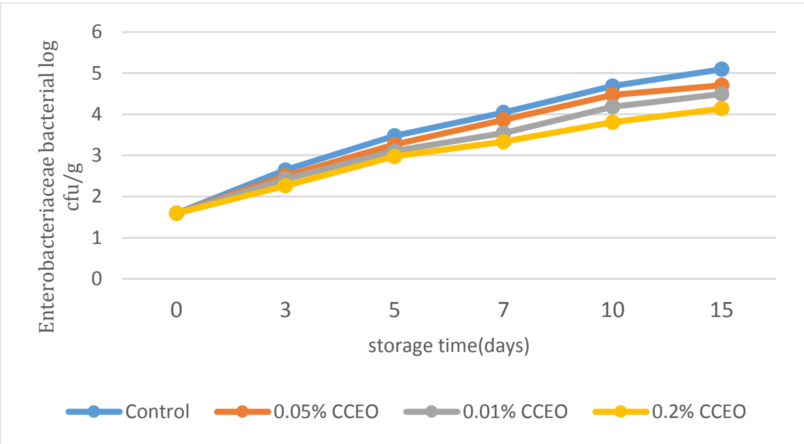

Figure 3. Changes in Enterobacteriaceae of Fresh camel sausage during refrigerated storage (*Cuminum cyminum* essential oil (CCEO))

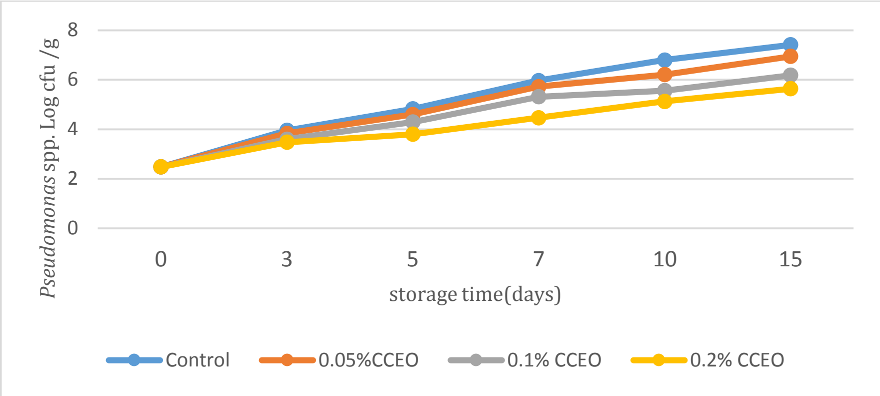

Figure 4. Changes in *Pseudomonas* of Fresh camel sausage during refrigerated storage (*Cuminum cyminum* essential oil (CCEO))

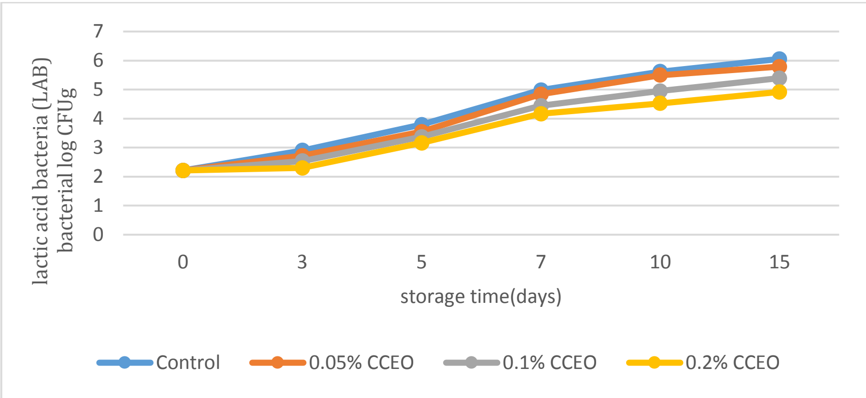

Figure 5. Changes in Lactic acid bacteria (LAB) of Fresh camel sausage during refrigerated storage (*Cuminum cyminum* essential oil (CCEO))

Low refrigeration temperatures increase the growth rate of psychrotrophic bacteria (PSB) in food products such as fresh sausage. At the beginning of the present study, PSB in the fresh camel sausage was estimated at 3.17 log CFU/g, which reached 9.48 log CFU/g, at the end of day 15 of storage in the control samples (Figure 2). In addition, an approximate reduction of 1-2 log CFU/g was observed in the PSB of the enriched samples with the highest concentrations of CCEO. This pattern is in line with the previous studies, indicating that the use of essential oils could decrease the growth of the PSB count in minced meat and meat products in chilled conditions (7, 22, 24).

Enterobacteriaceae have extensively been used as an indicator of the hygienic quality of food production. In the current research, the *Enterobacteriaceae* counts of the sausage samples continuously increased during the storage period within the range of 4.13-5.09 log CFU/g at the end of the study (Figure 3). Furthermore, the added CCEO caused a significant reduction in the *Enterobacteriaceae* counts of the fresh sausage samples (P<0.05). Our findings regarding the use of essential oils in fresh sausage are consistent with previous studies (7, 23).

The spoilage potential of *Pseudomonas* spp. in raw meat and meat products is associated with their ability to grow and produce hydrolytic enzymes (25) (26). In the present study, the initial count of *Pseudomonas* spp. was 2.48 log CFU/g, which reached 7.41 log CFU/g in the control samples on the last day of storage (Figure 4). The lowest *Pseudomonas* spp. count was recorded in the samples integrated with 0.2% CCEO (5.63 log CFU/g) after 15 days of storage. These findings are in line with the previous studies reporting a diminution in *Pseudomonas* count in fresh sausage enriched with various essential oils during refrigerated storage (7).

The contribution of lactic acid bacteria (LAB) to the spoilage of meat and meat products has been well documented (27). In the present study, the LAB count increased in the control samples from an initial count of 2.21 log CFU/g to 6.05 log CFU/g at the end of the chilled storage period (Figure 5). This is in line with the results obtained by Da Silveira et al. (2014), which indicated a significant reduction in the LAB count of Tuscan sausage after adding bay leaf essential oil (0.05% and 0.1%) (24). Regarding the use of essential oils, the results of the present study are consistent with the study by Ajourloo et al. (2020), which showed that the LAB count of Balkan-type sausage significantly decreased by the combination of MAP (20% CO_2 and 80% N_2) combined with *Ziziphora clinopodioides* essential oil compared to control samples (7).

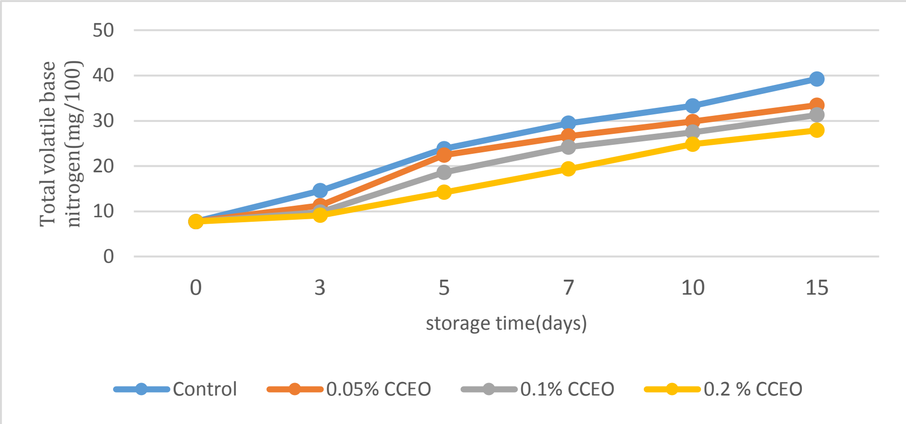

Figure 6. Changes in total volatile base nitrogen (TVB-N) value of Fresh camel sausage during refrigerated storage (*Cuminum cyminum* essential oil (CCEO))

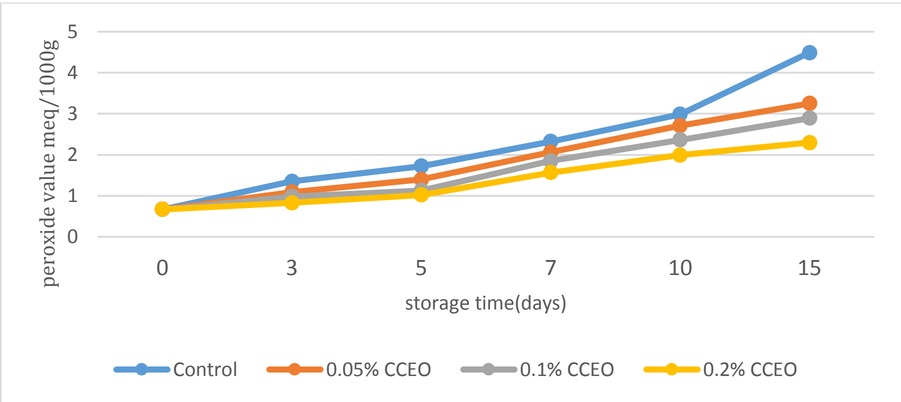

Figure 7. Changes in peroxide value of Fresh camel sausage during refrigerated storage (*Cuminum cyminum* essential oil (CCEO))

In another study, Badia et al. (2019) reported the initial count of LAB in Tuscan sausage to be 2.46-3.13 log CFU/g depending on the treatments and after the integration of 0.2% and 0.4% oregano and rosemary essential oils, which led to the significant extension of the shelf life of these samples compared to the controls (28). Compared to control samples, the integration of CCEO with the sausage formulation in the present study significantly retarded the growth of MBC, PSB, *Pseudomonas* spp., LAB, and *Enterobacteriaceae* family, in the fresh camel sausages. This could be attributed to the antimicrobial activity of the components in CCEO, including γ-terpinene, p-cymene, pinene, cuminaldehyde, safranal, and cuminal (12, 13).

Chemical Analysis

The TVB-N content of meat is an important biochemical biomarker of fresh proteinaceous products, which is associated with the protein degradation of meat (12). In the present study, a significant increase was observed in the TVB-N values of all the treatments by increasing the storage days (P<0.05). However, the integration of CCEO resulted in a slower rate of increase compared to the control samples. The initial TVB-N content was estimated at 7.75 mg/100 g, which reached 39.75 mg/100 g in the control samples at the end of the storage period. At the end of the study period, a significant reduction (approximately 6.29-11.85 mg/100 g) was observed in the final TVB-N of the samples integrated with CCEO compared to the control samples (P<0.05). This is in line with the previous studies reporting a reduction in TVB-N after the integration of essential oils during refrigerated storage (7, 23).

Fresh sausage is susceptible to lipid oxidation due to its high fat content, and the measurement of the peroxide value (PV) is a common method to assess the oxidation of fresh sausage (7) (29). In the present study, the initial PV of the control samples was estimated at 0.67 meq/1000 g of lipid. At the end of the study period, PV reached 4.49, 3.25, 2.89, and 2.23 in the control samples, and samples containing 0.05%, 0.1%, and 0.2% CCEO, respectively. This is also consistent with the previous studies focused on the impact of herbal extracts and essential oils on the reduction of lipid oxidation in fresh sausage (23, 30-32). The antioxidant activity of CCEO could be associated with radical-mediated lipid peroxidationit inhibition through scavenging hydroxyl and peroxy free radicals (33).

Sensory Evaluation

The color of meat and meat products is widely considered by consumers to determine the freshness and acceptability of these food products. In the present study, changes in the sensory properties of the fresh sausage samples containing 0.05% and 0.1% CCEO were minor. However, the integration of 0.2% CCEO caused a negative influence on the odor and taste indices of the samples. Furthermore, the higher production rate of biogenic amines, ketones, and aldehydes in the control samples led to lower scores by the panelists. These findings are in line with the study by Ajourloo et al.,(2020), which indicated the higher scores of the treated sausage samples with *Ziziphora clinopodioides* essential oil compared to the control samples (7).

Conclusion

According to the results, the main compounds of CCEO collected from Kerman province, Iran were 1,4-p-menthadiene-7-al (32.20%) and cuminaldehyde. It could be concluded that CCEO is a natural preservative that could effectively increase the shelf life of fresh camel sausage during storage in refrigerator conditions.

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