Journal of Food Measurement and Characterization (2021) 15:5231–5244
https://doi.org/10.1007/s11694-021-01096-1

ORIGINAL PAPER

Impact of zein coating impregnated with ginger extract and *Pimpinella anisum* essential oil on the shelf life of bovine meat packaged in modified atmosphere

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Received: 30 May 2021 / Accepted: 31 July 2021 / Published online: 6 August 2021

Abstract

In the present study, we aimed to evaluate the impact of zein coating (Z) impregnated with ginger extract (GE) and *Pimpinella anisum* essential oil (AEO) on microbial, chemical and sensory characteristics of beef samples packaged in modified atmosphere (MAP). The treatments were assessed during refrigerated storage for 20 days. GC-MS analysis exhibited that anethole (78.39%) was the dominant component of GE. Results indicated that meat treatment with zein coating containing GE 3% and AEO, resulted in the highest reduction rate of mesophilic bacteria (4.13 log CFU/g), lactic acid bacteria (2.68 log CFU/g), *Enterobacteriaceae* (3.84 log CFU/g), *Pseudomonas spp.* (2.74 log CFU/g), and yeasts–molds (1.99 log CFU/g) as compared to the untreated (control) samples. Moreover, the antimicrobial activity of GE enhanced with increasing the concentration and a synergistic effect was observed when combined with AEO. Chemical properties including total volatile nitrogen, thiobarbituric acid reactive substances (TBARS), peroxide value (PV) and pH were notably lower in all treated meats compared to control samples. However, PV and TBARS were higher in MAP only treated meats. Regarding organoleptic properties, Z-GE 3%-A was mostly acceptable the panelists until the last day of experiment. Our findings indicate the potential of zein edible coating incorporated with GE and AEO in reducing the deterioration of fresh beef and improving its shelf life under MAP conditions.

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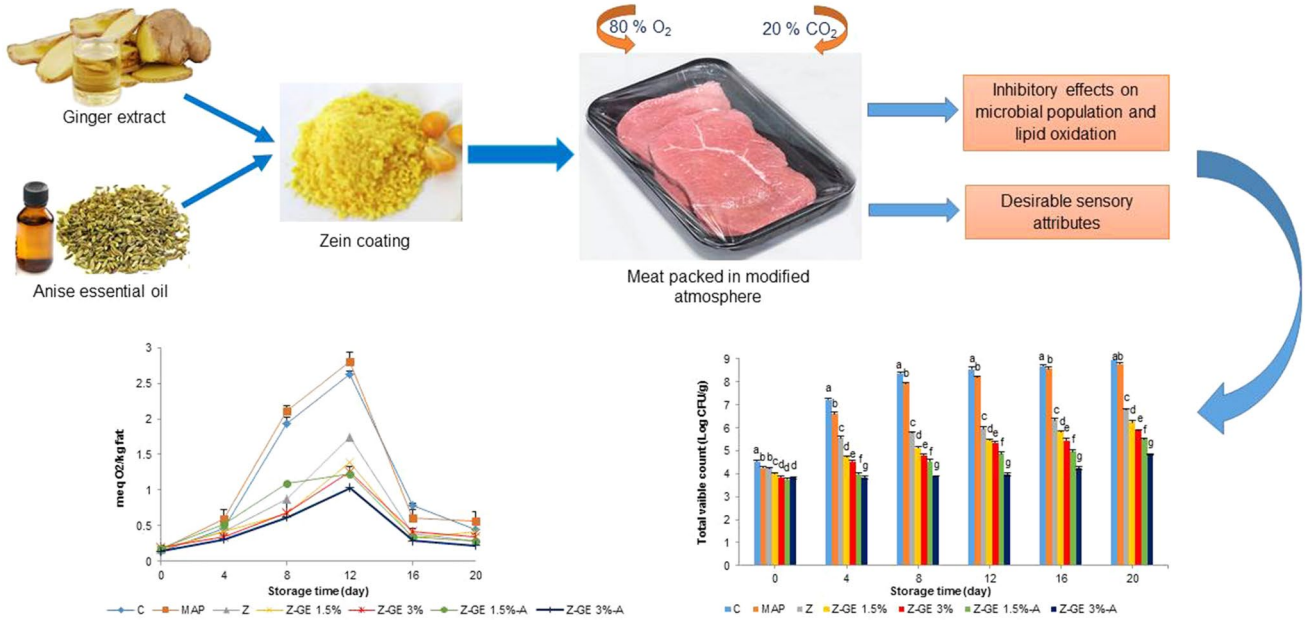
Graphic abstract



Keywords Fresh beef · Zein · Ginger extract · *Pimpinella anisum L.* · Antimicrobial properties · Modified atmosphere packaging

Introduction

In the last decade, consumers' desire for better, fresher, and easier-to-access food products has increased. Microbial growth as well as oxidative and chemical changes are the most important factors reducing the quality of food products during storage. According to the Centers for Disease Control and Prevention (CDC), 48 million people in the United States are diagnosed with foodborne illness each year, of which 3000 have died and 128,000 have been hospitalized [1]. Generally, meats and meat-based foods have very low oxidative and microbial stability due to their high moisture and protein contents. So, they can be easily affected by chemical and microbial spoilage during the storage period [2]. The most important way to inhibit the growth of spoilage and pathogenic microorganisms in meat is to use chemical preservatives. Despite several approved and commercialized synthetic antimicrobials, their application is limited. Studies have shown that some cancers may be associated with chemical preservatives and their toxic residues [3]. Another method to enhance the shelf life of meat is the use of synthetic coatings, which are sometimes less accepted by consumers due to side effects such as carcinogenic and teratogenic impacts [4]. Therefore, there is a greater tendency to use edible coatings enriched with essential oils and extracts with natural origins. Edible coatings are thin layers of biodegradable materials that cause physical protection and also maintain and improve the quality level of foods [5–8].

Zein contains a group of prolamines found in corn endosperm cells and makes up more than 50% of total proteins [9]. Zein has unique properties for preparing films and edible coatings due to its high percentage of non-polar amino acids as compared to other plant proteins. Ability to form excellent coatings and films, solubility in organic solvents (ethanol), compatibility with antimicrobial and antioxidant compounds are among the properties of zein biopolymer which make it possible for its application in fresh meat packaging [10].

Ginger is a traditional condiment which derives from the roots of the plant called *Zingiber officinale.* It has a medicinal properties and widely used for treatment of various ailments including nausea, asthma, colds, migraines, hypertension and arthritis [11]. Ginger can be used as a natural antioxidant due to its volatile and non-volatile antioxidant compounds in different parts, especially in the rhizome [12]. In addition, the phytochemical components of this spice have been investigated and its antimicrobial activities against several pathogens have been proven [13].

Pimpinella anisum L. is an annual herb, commonly known "Aniseed or Anise" and belongs to family Apiaceae. This plant has small green to yellow seeds and white flowers

that grows in regions such as Iran, Egypt, India, Turkey and other tropical areas [14]. Cultivation of this plant is important for the production of aromatic seeds, which is widely used in pharmaceutical and food industries. The essential oil from Anise can be used as a flavoring agent in food products and also as carminative, analgesic in migraine, relief of gastrointestinal spasms, disinfectant and digestive [15].

There are several types of packaging for enhancing the shelf life of food, especially meat products. One of the best types of packaging is Modified Atmosphere Packaging (MAP). In this packaging system, a mixture of gases such as carbon dioxide, oxygen and nitrogen is used. Some factors, such as color changes, lipid oxidation and microbial contamination can adversely affect the durability of red meat during refrigerated storage and reduce its quality. However, manipulation of gases inside the red meat packaging has been shown to be effective in preventing these factors [3].

Numerous studies have reported the antioxidant and antimicrobial properties of ginger and aniseed [13, 16–18]. However, there is a dearth of study on the combined use of ginger extract and aniseed essential oil in a biodegradable film and checking their functionality in a real food system. Due to the importance of red meat in different food cultures around the world, it is necessary to study different methods to increase the shelf life of this valuable food. Hence, the current study aimed to investigate the efficacy of zein edible coating enriched with ginger extract and *Pimpinella anisum* essential oil on the shelf life of red meat in MAP conditions during the refrigerated storage period.

Materials and methods

Preparation of ginger extract (GE)

Ginger was purchased from local groceries in Shiraz, Iran. Its root was cut into pieces, washed and dried under shade. The dried roots were ground into powder in a kitchen blender (Bosch, Germany) and afterwards passed through a 60-mesh sieve. Subsequently, 100 g of powdered ginger was added to 1 L alcohol (ethanol) and shaking with a round of 150 rpm for 48 h at 25 °C. The obtained mixture was then filtered (sterile Whatman No. 1 filter paper) and placed in the desiccator for final concentration. The obtained extract was stored in a refrigerator in a light and air-insulated glass until use [19].

Preparation and analysis of anise essential oil (AEO)

Anise seeds were achieved from Qasr-e Dasht garden, Shiraz city, Fars province, Iran (29.64 N, 52.48 E). For essential oil extraction, dried and powdered seeds were hydrodistillated for 3 h by Clevenger equipment and the obtained AEO was dried with anhydrous sodium sulfate. Then, AEO was passed through 0.22 µm sterile filters and stored in a dark container at 4 °C until experiments. For identification of AEO chemical compositions, gas chromatography-mass spectrometry (GC-MS) system (GC-MS; 6890 N, Agilent Technologies, Palo Alto, CA, USA) was used. The GC-MS conditions were implemented according to the study of Khanjari et al. [20]. The obtained peaks form the GC chromatogram was identified by comparing with pure compounds kept in the literature and the National Institute of Standards and Technology (NIST 08) library.

Preparation of zein coating solution

The zein (Z) powder (5.4 g), citric acid (1 g), and glycerol (1.4 mL) as plasticizer were mixed in 95% ethanol (52 mL) by using a magnetic stirrer until entirely dissolved and a uniform solution achieved [21, 22].

Meat samples treatment

Fresh bovine meat was used for the experiments. Meat samples were purchased from a local butcher shop of Shiraz and after deboning, they were transferred to the laboratory by an insulated ice flask. Meats were cut uniformly into steak size (2.53 cm thickness) and kept at refrigerator until experiments. Samples were separated into 7 groups and coated as follows: control (without Z-GE-A-MAP), MAP (without Z-GE-A), Z, Z-GE 1.5%, Z-GE 3%, Z-GE 1.5%- A 1% and Z-GE 3%- A 1%. Meat samples were immersed in coating solutions (2 min), drying well at 10 °C and then, packed in sterile polyethylene bags under modified atmosphere. MAP conditions were provided using MAP machine (A200 Model; Henkelman, Germany) by flushing the packages with the required gases with compositions of 20% CO_2 and 80% O_2. Afterwards, treated samples were stored in a refrigerator for further quality evaluations [3, 23].

Microbial assessments

Microbial evaluation of treated samples was performed on days 0, 4, 8, 12, 16 and 20 of storage. A quantity of 10 g of meat sample was weighed in a sterile zipper bag and 90 mL of sterile peptone water (0.1%) (Merck, Darmstadt, Germany) was added and homogenized with a stomacher blender (Seward, London, UK) for 3 min. After appropriate serial dilutions, 0.1 mL of the prepared dilutions was transferred to desired culture media for microbial enumeration. For mesophilic total viable counts (TVC), plate count agar (Merck Millipore) was applied and incubation was done at 30 °C for 2 days. Lactic acid bacteria (LAB) were counted in de Man Rogosa Sharpe (Merck, Darmstadt, Germany, Oxoid) agar after incubation for 2 days at 30 °C

under anaerobic conditions. Populations of *Pseudomonas spp.* were enumerated on Pseudomonas agar base supplemented with cephaloridine, fucidin, and cetrimide (CFC) and incubated at 25 °C for 2 days. *Enterobacteriaceae* bacteria were enumerated on Violet Red Bile Glucose (VRBG) agar (Merck Millipore) (incubated for 24 h at 37 °C). Yeast-molds were identified in Rose Bengal chloramphenicol selective agar (Merck, Darmstadt, Germany) followed by incubation for 3–5 days at 25 °C. All microbial counts were expressed as log CFU/g. [14, 21, 24].

TVN determination

Total Volatile Nitrogen (TVN) of meat samples was measured by macro Kjeldahl procedure according to the method of Jouki et al. [25]. Briefly, 10.0 g of homogeneous meat was mixed with 3.0 g MgO and then distilled with 300.0 mL of distilled water. After titration by sulphuric acid solution, the value of TVN was reported as mg TVN per 100 g of meat.

TBARS determination

To evaluate the lipid oxidation changes of treated meats, thiobarbituric acid reactive substances (TBARS) experiment was used according to the method of Pikul et al. [26] with some modifications. Sample (10.0 g meat) was mixed with 35 mL 5% (v/v) trichloroacetic acid (TBA) and 1 mL butylated hydroxytoluene (1 mg/mL). After filtering the mixture (Whatman No. 1 filter paper), 5 mL of filtered solution and 5 mL of TBA was inserted into flask and incubated in water bath at 80 °C for 90 min. Finally, the absorbance of solution was measured at 532 nm and the TBARS value was recorded as mg MDA/kg meat.

PV determination

Peroxide value (PV) of samples was measured according to the International Dairy Federation (IDF) method [27]. Approximately 9.8 mL of chloroform-methanol (Merck, Germany) was added to 0.2 g of meat samples and stirred for 4 s. Afterwards, 10 mmol L^{-1} ammonium thiocyanate (Merck, Germany) was added to the mixture. Then, iron solution was added and kept at room temperature for 5–10 min. Eventually, the absorbance of prepared solutions was determined at 500 nm and the PV was reported as meq O_2/kg fat.

pH measurement

About 5.0 g of each treated meat was homogenized in distilled water (25 mL) for 1–2 min. Then, the pH of homogenized samples was measured at 25 °C by a digital pH meter (Mi 150, Milwaukee, Italy) [28].

Sensory evaluation

Sensory evaluation of quality of raw and cooked beef meat (taste, odor, color, texture and overall acceptability) was performed according to the method of Petrou et al. [29]. Evaluation of cooked meats was performed on day 0 and cooking the meats was done by placing the samples on a flame at a distance of 10 cm (10 min) until the final central temperature of the meat reached to 86 °C. Sensory characteristics of treated meats were carried out by thirty semi-trained panelists using 9-point hedonic scale (1: too poor, 9: too good). All participants signed Informed Consent Forms (ICM) and the work was approved by the Ethics Committee of Fasa University of Medical Sciences, Fasa, Iran (Ethical approved number: IR.FUMS.REC.1399.130).

Statistical analysis

All experiments were carried out in three replications (mean ± SD). Data analysis was performed using SPSS statistical software, (release 23.0) for Windows (SPSS Inc., Chicago, IL, USA). Statistical analysis of data was performed using one-way analysis of variance (ANOVA) and significant differences were carried out by Duncan's multiple range tests. Significance level was set for $P < 0.05$.

Results and discussion

Chemical constituents of anise essential oil

To determine the antimicrobial efficacy of EOs, it is important to identify their chemical constituents. The major chemical composition of AEO is presented in Table 1. Overall 20 compounds were detected which representing 98.96% of anise EO. The results of GC-MS exhibited that anethole (78.39%) was the dominant component, followed by piperitenone oxide (7.37%), thymol (4.74%), estragole (2.46%), p-allylanisole (1.28%) and α-thujone (1.03%). These results are consistent with the findings of Khanjari et al. [20] and Abdel-Reheem and Oraby [18] reporting anethole (80.84%, 79.62%) as the main constituent of AEO. The difference in the proportion of the main components can be related to the genetic variation, soil composition, climatic conditions, and the harvesting stage of the plant that markedly affect the quality and quantity of AEO. It has been indicated that harvesting at early waxy stage and warm weather lead to higher quality and good yield of EO, respectively [30, 31].

Microbiological changes

The impacts of different treatments on microbial changes (population of total viable bacteria, LAB,

Table 1 Major chemical composition of anise essential oil

Compound	RTa (MIN)[a]	Percent[b]
Anethole	19.516	78.39
Piperitenone oxide	20.713	7.37
Thymol	21.679	4.74
Estragole	15.325	2.46
p-Allylanisole	14.236	1.28
α-thujone	11.574	1.03
Trans-Caryophyllene	22.762	0.73
Pulegone	13.852	0.64
Caryophyllene	22.431	0.59
β-Myrcene	14.716	0.37
β-Pinene	11.732	0.26
Camphene	17.338	0.24
trans-β-Farnesene	26.528	0.19
Menthone	14.461	0.17
α-Humulene	21.479	0.13
Spathulenol	29.582	0.11
3-octanol	22.259	0.09
Pinene -α	11.563	0.07
2-β-Pinene	6.347	0.05
Germacrene-D	13.582	0.05
Total identified		98.96

[a]Retention time

[b]Relative proportions as percent of the total peak area

Enterobacteriaceae, *Pseudomonas spp.* and yeasts–molds) of fresh meats are depicted in Figs. 1, 2, 3, 4 and 5. The TVC value of samples was between 3.81 and 4.52 on the initial day, which indicated a notable difference between the control and the treated samples ($P < 0.05$). These results represent a strong antimicrobial property for coating compounds. The amount of TVC in all groups was significantly increased over time ($P < 0.05$). The lowest TVC was reported in Z-GE 3%-A group and the highest TVC was found in the control samples at the end of the storage. The maximum acceptable level of TVC for MAP treated meats is recommended less than 7 log CFU/g [32]. In control group, TVC increased to 7.23 log CFU/g after 4 days of storage and reached to 8.97 log CFU/g on the 20th day. In addition, for MAP samples, the TVC value exceeded the acceptable limit after 8 days of storage. Our results are similar to Murphy et al. [33] findings, indicating the impact of MAP on controlling the microbial growth. The average TVCs of coating treated samples were in the following order: Z > Z-GE 1.5% > Z-GE 3% > Z-GE 1.5%- A > Z-GE 3%- A. These findings indicate that zein coating has antimicrobial properties and increases when combined with plant extracts or essential oils [21]. Moreover, the antimicrobial activity of ginger extract enhanced with increasing the concentration and a synergistic effect was observed when combined with anise EO. The strong antimicrobial properties of ginger are related to more than 400 different compounds, including: zingerone, gingerols, shogaols and sesquiterpenoids [13]. The inhibitory effect of anise EO is related to the hydroxyphenolic groups,

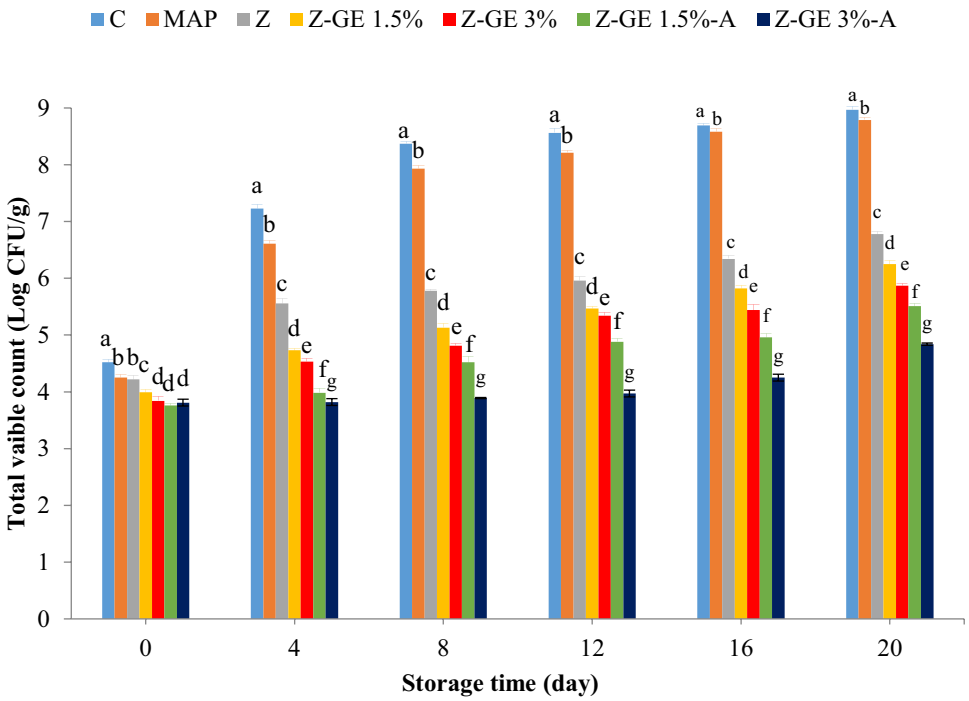


Fig. 1 Alterations in mesophilic total viable counts (log CFU/g) of beef samples packed in modified atmosphere during the refrigerated storage at 4 °C. Error bars display the standard deviation of TVC for each treatment during 20 days of experiment. *C* control (without Z-GE-A-MAP), *MAP* modified atmosphere packaging (without Z-GE-A), *Z* zein coating, *Z-GE 1.5%* zein with ginger extract 1.5%, *Z-GE 3%* zein with ginger extract 3%, *Z-GE 1.5%-A* zein with ginger extract 1.5% and AEO, *Z-GE 3%-A* zein with ginger extract 3% and AEO. The same lowercase letters are not significantly different between various treatments within the same study day at $P > 0.05$

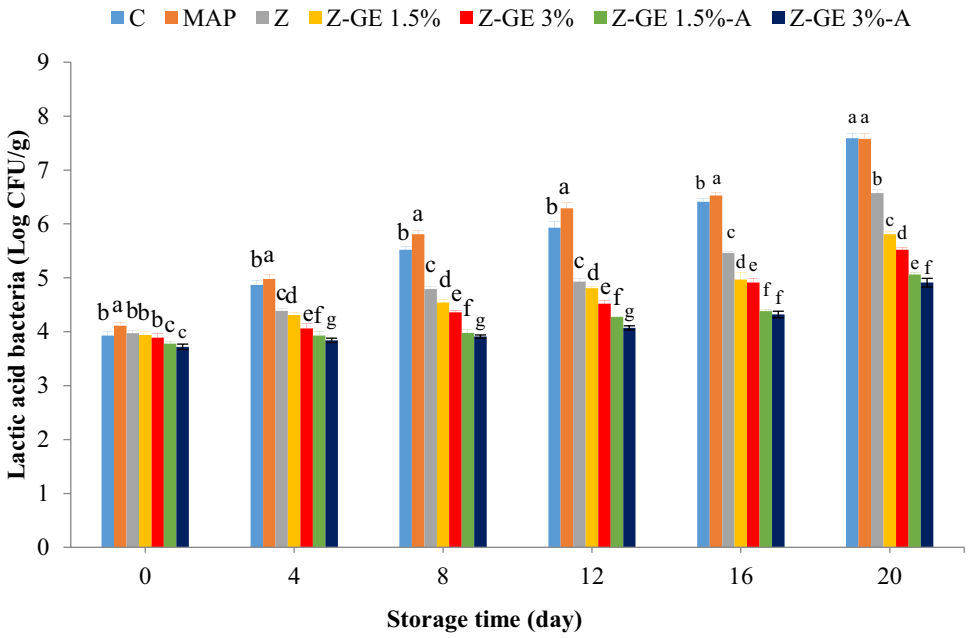


Fig. 2 Alterations in counts of lactic acid bacteria (log CFU/g) in beef samples packed in modified atmosphere during the refrigerated storage at 4 °C. The same lowercase letters are not significantly different between various treatments within the same study day at $P > 0.05$

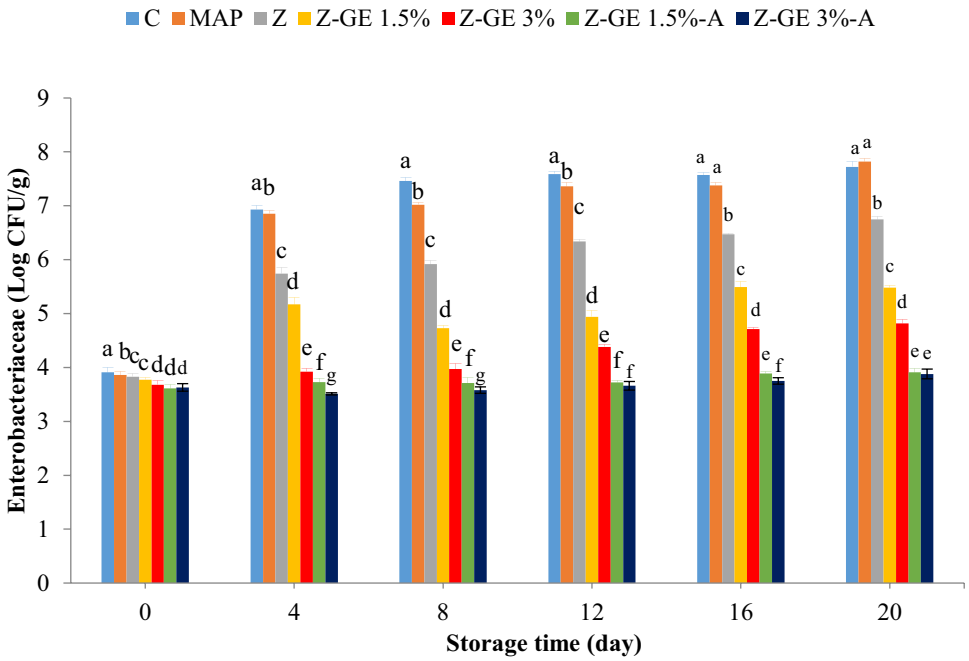


Fig. 3 Alterations in counts of *Enterobacteriaceae* (log CFU/g) in beef samples packed in modified atmosphere during the refrigerated storage at 4 °C. The same lowercase letters are not significantly different between various treatments within the same study day at $P > 0.05$

which react with proteins in the bacterial cytoplasmic membrane and cause changes in the membrane permeability. These reactions lead to the formation of pores and ultimately affect the protons flow [14]. Similar results were achieved by Wang et al. [34] that EOs of ginger and cinnamon incorporated in chitosan edible film notably inhibited the total bacterial growth in pork slices in comparison with the control (unwrapped) samples. Mitsumoto et al. [35] showed that the suitable shelf life of raw beef at 4 ° C is about 7 days, while in the present study it was found that the treated samples have a good microbiological quality even up to the 20th day of storage.

Lactic acid bacteria are facultative bacteria which are cultured anaerobically. These organisms are the natural and main microflora of fresh meat which can prevent the growth of spoilage bacteria and even pathogens [36]. According to the Fig. 2, the initial amount of LAB was 3.93 log CFU/g in control group and the population increased to 7.59 log CFU/g at the end of the cold storage. For treated samples, the number of LAB significantly increased ($P < 0.05$) during

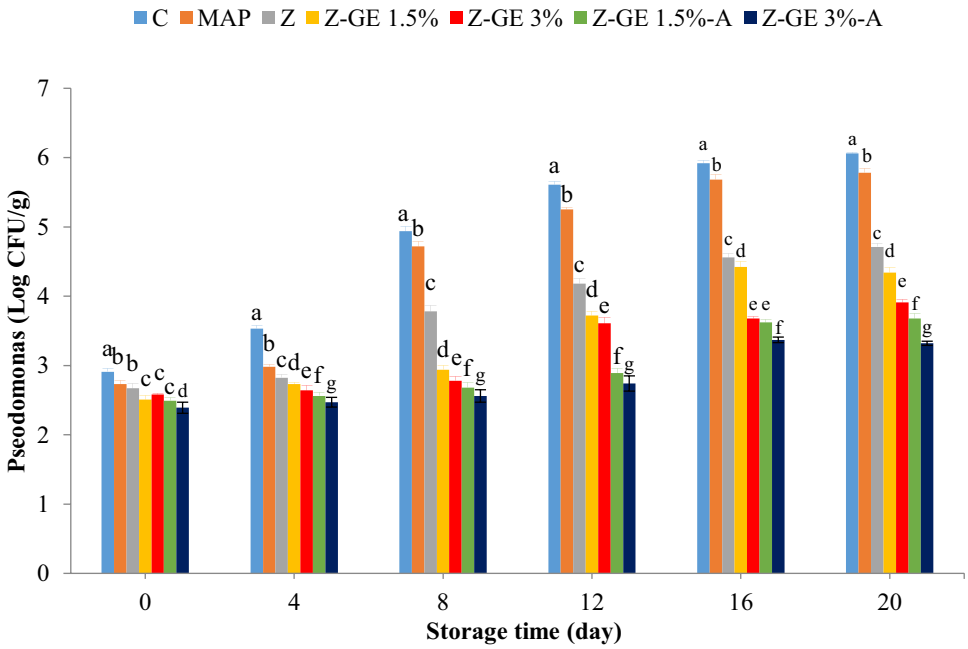


Fig. 4 Alterations in counts of *Pseudomonas* spp (log CFU/g) in beef samples packed in modified atmosphere during the refrigerated storage at 4 °C. The same lowercase letters are not significantly different between various treatments within the same study day at $P > 0.05$

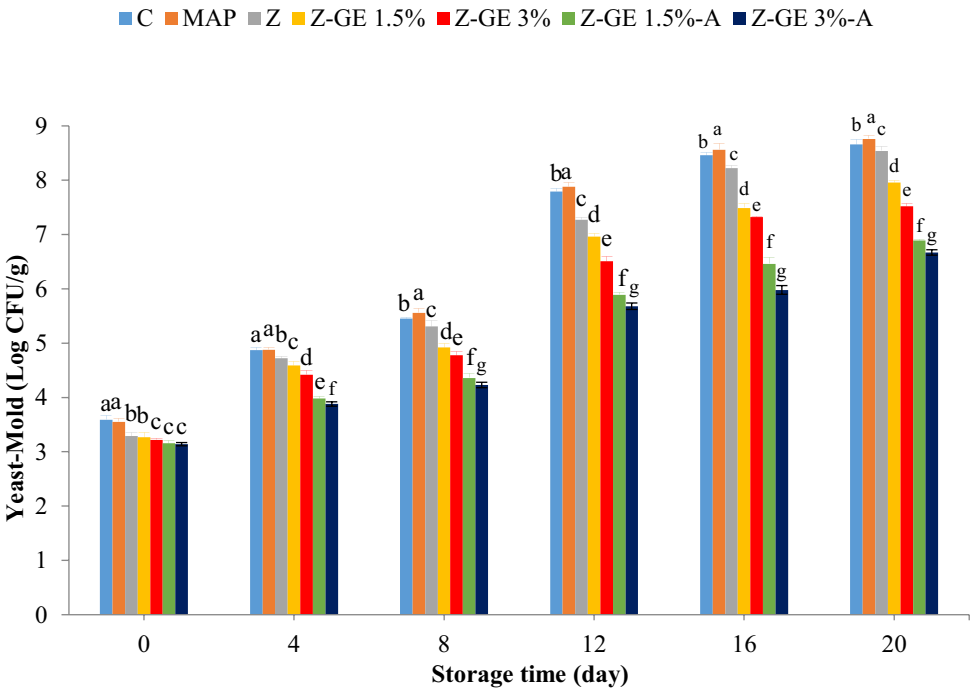


Fig. 5 Alterations in counts of yeasts–molds (log CFU/g) in beef samples packed in modified atmosphere during the refrigerated storage at 4 °C. The same lowercase letters are not significantly different between various treatments within the same study day at $P > 0.05$

the storage period and counts for Z, Z-GE 1.5%, Z-GE 3%, Z-GE 1.5%- A and Z-GE 3%-A reached 6.57, 5.81, 5.52, 5.06 and 4.91 log CFU/g, respectively, representing lower population compared with the control sample. In a study conducted by Khanjari et al. [20], they observed that the growth of gram-positive LAB in minced beef adversely affected by the increasing the concentration of *Pimpinella anisum* EO. In another research, Tajik et al. [37] revealed that the population of LAB reached from 3.05 to 5.95 log CFU/g for patties treated with 0.1% *Zataria multiflora* Boiss EO and 0.1% extract of grape seed at 9th day of storage.

Enterobacteriaceae are a large group of gram-negative microorganisms which are considered as hygiene indicator organisms [38]. The primary population of *Enterobacteriaceae* was 3.91 log CFU/g, representing the appropriate quality of beef and reaching to the amount of 7.72 log CFU/g in control group at the end of the storage period. Similar findings were achieved by Keykhosravy et al. [39].

The assessment of treated meats exhibited that Z-GE 1.5%-A (3.91 log CFU/g) and Z-GE 3%-A (3.88 log CFU/g) had the lowest number of *Enterobacteriaceae* on the final day of cold storage (Fig. 3), representing a reduction of about 3.82 log cycles compared with the untreated (control) group. Fewer counts of *Enterobacteriaceae* in the combined treatment of zein with GE and anise EO revealed the synergistic impact compared with Z-GE groups. Similarly, Mayeli et al. [21] reported a reduction of 1–5 log cycles in population of *Enterobacteriaceae* when zein coating was used in combination with sour orange peel extract for rainbow trout samples. Studies attributeibute the antimicrobial properties of anise EO to the presence of different compounds including terpene hydrocarbons and anethole [18, 20]. In addition, the inhibitory impact of zein film in the absence of antibacterial compounds has been attributed to the alcohol used in the formulation of film [40].

Pseudomonas species are gram-negative and obligatory aerobic bacteria which have been identified as spoilage organisms in meats stored in the refrigerator. These organisms, by producing proteases, lead to food proteolysis and spoilage [41]. The primary number of *Pseudomonas* spp. was ranged from 2.39 log CFU/g in Z-GE 3%-A to 2.91 log CFU/g in control samples. According to the data, treatment with GE and anise EO exhibited the inhibitory impact on the counts of *Pseudomonas* spp. throughout the storage time. The final population of *Pseudomonas* spp. in MAP, Z, Z-GE 1.5%, Z-GE 3%, Z-GE 1.5%- A and Z-GE 3%-A samples reached 5.78, 4.71, 4.34, 3.91, 3.68, 3.32 log CFU/g, respectively as compared with control sample (6.06 log CFU/g) (Fig. 4). As can be observed, meats treated with Z-GE 3%-A showed the lowest count of *Pseudomonas* spp. (2.74 log cycles decrease) in comparison with controls on the last day of storage. It was demonstrated that gas composition including $CO_2/O_2/N_2$ 30/70/0 and $CO_2/O_2/N_2$ 30/50/20 effectively prevent the growth of *Pseudomonas* spp. in minced beef packaged by MAP method [42]. Our results are in accordance with the report of Kačániová et al. [43] who indicated that natural products derived from plants can be effective for preventing the growth of *Pseudomonas* spp.

Fungi (molds-yeasts) are a group of microorganisms which can grow on almost any type of food material and cause different degrees of decay and deterioration. Some fungal species may be dangerous to human health as they can produce toxic compounds called mycotoxins. The population of molds-yeasts at day 0 (Fig. 5) was 3.59 log CFU/g in control samples. The count of yeasts-molds was increased over time and MAP group (8.76 log CFU/g) exhibited higher counts compared with control group (8.66 log CFU/g) on the 20th day, which is in line with the study of Langroodi et al. [3]. At the end of the storage period, the counts of yeasts-molds was 8.54 log CFU/g in Z group, followed by Z-GE 1.5% treatment (7.96 log CFU/g), Z-GE 3% (7.52 log CFU/g), Z-GE 1.5%- A (6.89 log CFU/g) and the lowest number was observed in Z-GE 3%-A samples (6.67 log CFU/g). The antifungal impact of anise EO has been demonstrated by Özcan and Chalchat [43] against various fungi. The authors showed that the phenolic components in EOs have the highest antifungal activity, followed by ketones, alcohols and aldehydes. Moreover, the antifungal properties of ginger have been previously reported [13]. Similarly, the combination of *Bunium persicum* Boiss with *Zataria Multiflora* Boiss has been revealed to be effective in preserving the turkey meat [39]. Hence, the obtained results indicated that the application of biodegradable zein-based coating enriched with ginger extract and anise EO could prevent the growth and proliferation of spoilage microorganisms and pathogens.

pH value

The alterations in pH of meat samples throughout refrigerated storage are presented in Fig. 6. As indicated, the pH of all samples markedly increased ($P < 0.05$) during the refrigeration period for 20 days. The initial pH of meat samples ranged from 5.82 to 5.18, in which meats with zein coating impregnated with GE or GE-A exhibited the lowest levels of pH. These results are in accordance with the study of Zakrys et al. [44]. Among various treatments, Z-GE 3%-A treatment had the best impact which eventually reached the pH of 6.23 compared to the control group (8.18). The increase in the pH value of meat samples can be attributed to the decomposition of nitrogenous compounds and the accumulation of substances such as amines and alkaline NH_3 components due to the activity of microbial or endogenous proteases [45]. It has been reported that the rise of pH has a negative impact on the quality of meat, particularly in terms of sensory properties including color, texture and odor [46]. The lower pH in Z-GE 3%-A treatment shows the antimicrobial activity of GE and anise EO, which prevents the growth of spoilage microorganisms and consequently degradation of nitrogen compounds. This outcome is consistent with the results obtained from microbial analysis. Similar results in stewed-pork have been reported by Cao et al. [19]. Besides, the lower pH of MAP samples compared to the control group can be attributed to the dissolution of CO_2 in water and production of carbonic acid [47].

Peroxide value

Oxidation of lipids causes an unpleasant rancid flavors and toxic compounds, which leads to a decline in the product quality. In the current study, peroxide value was determined as an indicator of primary lipid oxidation. Figure 7 shows the alterations in PV in fresh meat samples during storage at 4 °C for 20 days. As can be seen, control and

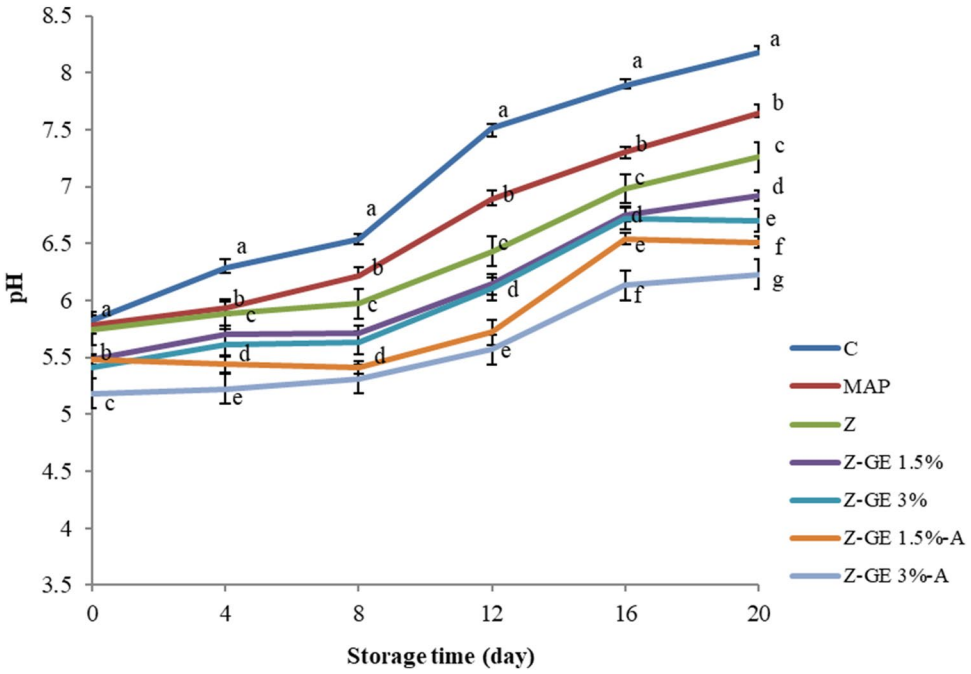


Fig. 6 Alterations in pH values of MAP-treated beef samples during the refrigerated storage at 4 °C. Error bars display the standard deviation of pH for each treatment during 20 days of experiment. The same lowercase letters are not significantly different between various treatments within the same study day at $P > 0.05$

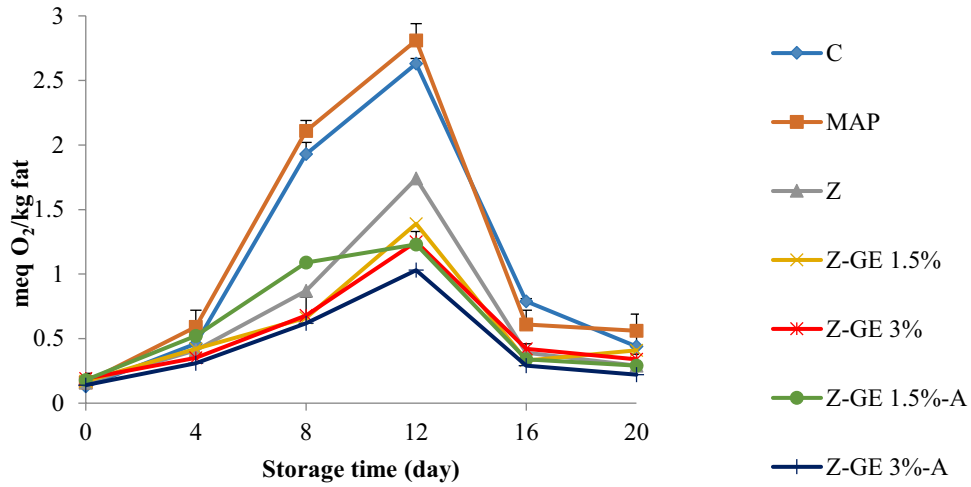


Fig. 7 Alterations in peroxide value (meq O_2/kg fat) of MAP-treated beef samples during the refrigerated storage at 4 °C. Error bars display the standard deviation of PV for each treatment during 20 days of experiment

MAP samples had higher PV ($P < 0.05$) compared to other treated meat samples throughout the refrigeration period. The initial amount of PV in control meats was 0.14 meq O_2/kg fat, enhanced to 2.63 meq O_2/kg fat till day-12 and afterwards decreased to 0.41 meq O_2/kg fat at the end of the storage (day 20). A similar trend was observed for all treated samples. The decrease in PV after 12 days of storage is possibly due to the decomposition of peroxide and synthesis of secondary lipid oxidation metabolites [20]. Our findings are consistent with Raeisi et al. [48] achievements for chicken meat coated with alginate containing rosemary and cinnamon EOs. The lowest PV was observed in Z-GE 3%-A treatment which implies the impact of ginger extract and anise EO on controlling the peroxide enhancement. Besides, the antioxidant activities of GE and anise EO are mostly related to their phenolic compounds, which can act as radical scavengers or potential hydrogen donors [49]. Anise EO has also been shown to markedly reduce peroxide value in minced beef [20]. It has been revealed that ginger contains various antioxidant components such as flavonoids, polyphenols and total tannin; therefore, the decrement of PV determined in treated samples is expected [12]. Meanwhile, with increasing the concentration of GE, the amount of PV decreased. It has been reported that zein film may contain some water soluble protein fractions that have antioxidant properties [40]. In the present study, MAP samples had higher PV than the control group, which is probably due to the high

content of oxygen in the MAP treatment. Similar results were reported by Hur et al. [50].

Assessment of total volatile nitrogen (TVN)

TVN is commonly considered as a criterion for meat freshness and quality. As a result of the activity of microorganisms and their destructive impact on food proteins, various volatile nitrogen compounds such as dimethylamine, trimethylamine and ammonia are produced [51]. The TVN content of fresh beef samples during refrigerated storage are depicted in Fig. 8. In all samples, the amount of TVN increased significantly ($P < 0.05$) during keeping time, and this was more prominent for untreated (control) meats, which was increased from 8.41 to 29.56 mg/100 g throughout the refrigerated storage. According to the Iran Veterinary Organization, the acceptable limit for TVN is considered 28 mg/100 g [52]. It is clear that the amount of TVN in the control samples exceeded the acceptable level at the end of the storage time, while in other treatments it is even in passable level until the last day of storage. Enhancing the concentration of GE significantly reduced the content of TVN and when incorporated with anise EO, their synergistic effect caused further TVN reduction. The best TVN result was recorded for Z-GE 3%-A treatment which reached to 14.16 mg/100 g on the last day of experiment. It has been shown that there is a direct correlation between TVN content and meat spoilage [53]. The antimicrobial impacts of zein coating, GE and anise EO on the level of microorganisms have been previously reported, which can be responsible for the identified differences between the control group and other treated samples [54]. Mayeli et al. [21] revealed that zein film alone and impregnated with sour orange peel extracts could markedly diminish the formation of TVN. This could be related to the inhibition of microbial growth and oxidation of lipids for the synthesis of TVN from amino compounds [55].

Assessment of TBARS

TBARS test is commonly used to measure the degree of lipid peroxidation, representing the amount of substances (mainly ketones and aldehydes) produced in secondary lipid oxidation. These by-products of oxidation lead to generate rancid aroma and off-flavor in oxidized meats and therefore adversely affect their sensory quality [56]. The impact of different treatments on TBARS level of meat samples during 20 days of cold storage are presented in Table 2. The amount of TBA at day 0 was 0.26 mg MDA/kg meat in control samples and increased to 2.83 mg MDA/kg meat at the end of the storage period. The obtained results indicated that the TBARS values markedly enhanced throughout the storage time for all meat samples ($P < 0.05$). According to the results, GE notably reduced the amount of TBA in treated meats and its effect was concentration-dependent. This means that by increasing the concentration of GE (from 1.5 to 3%) a greater decrease in TBA levels was observed. Fresh meats treated with high concentration of GE (3%) along with AEO, had the lowest TBA value (1.15 mg MDA/kg meat) after 20 days of storage. This is probably due to the synergistic impacts and potent antioxidant activity of plant compounds. Moreover, the content of TBARS in MAP treated meats was higher than untreated meats (controls). Similarly, Zakrys et al. [44] reported that there is a notable positive correlation between oxygen concentration and oxidation of lipids. Studies have shown that essential oils can neutralize the formation of free radicals by donating electrons [57]. It has been demonstrated that ethanolic and aqueous extracts of ginger possess potent antioxidant activity and inhibit lipid oxidation damage due to their radical scavenging properties [58]. Moreover, Amer and Aly [59] indicated that *Pimpinella anisum* L. had the capability to scavenge the DPPH radicals by about 91.3% which was close to the scavenging impact of ascorbic acid (95.3%). The outcomes of the present study are in accordance with the findings of Barkhordari

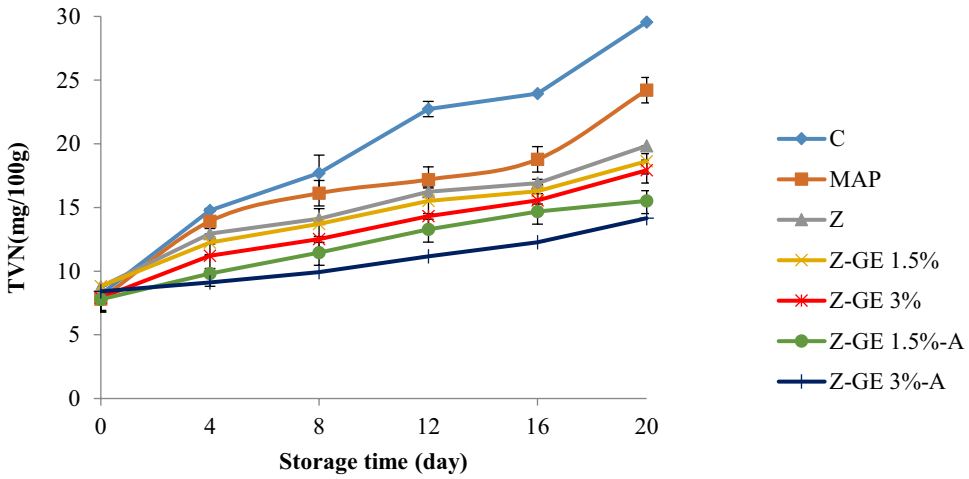


Fig. 8 Alterations in total volatile nitrogen (mg/100 kg meat) content of MAP-treated beef samples during the refrigerated storage at 4 °C. Error bars display the standard deviation of TVN for each treatment during 20 days of experiment

Table 2 Alterations in values of TBARS in MAP-treated beef samples during 20 days of refrigerated storage

Treatment	Storage time (day)					
	0	4	8	12	16	20
C	0.258 ± 0.04^{Ab}	0.649 ± 0.04^{Bb}	0.980 ± 0.05^{Cc}	1.279 ± 0.04^{Db}	2.346 ± 0.52^{Eb}	2.831 ± 0.05^{Fb}
MAP	0.236 ± 0.04^{Ab}	0.811 ± 0.04^{Ba}	1.257 ± 0.11^{Ca}	1.612 ± 0.19^{Da}	3.253 ± 0.07^{Ea}	3.941 ± 0.03^{Fa}
Z	0.280 ± 0.05^{Ab}	0.512 ± 0.71^{Bb}	1.231 ± 0.08^{Ca}	1.357 ± 0.15^{Cab}	2.328 ± 0.17^{Db}	2.821 ± 0.05^{Eb}
Z-GE 1.5%	0.279 ± 0.04^{Ab}	0.630 ± 0.12^{Bb}	1.119 ± 0.05^{Ca}	1.226 ± 0.17^{Db}	1.517 ± 0.03^{Ec}	2.443 ± 0.14^{Fb}
Z-GE 3%	0.325 ± 0.07^{Aa}	0.651 ± 0.11^{Bb}	1.020 ± 0.09^{Cb}	1.110 ± 0.15^{Cb}	1.471 ± 0.23^{Dc}	1.623 ± 0.03^{Ec}
Z-GE 1.5%-A	0.330 ± 0.05^{Aa}	0.621 ± 0.03^{Bb}	0.820 ± 0.03^{Cc}	0.862 ± 0.10^{Cc}	1.236 ± 0.19^{Dcd}	1.42 ± 0.06^{Ecd}
Z-GE 3%-A	0.326 ± 0.09^{Aa}	0.391 ± 0.09^{Ac}	0.721 ± 0.03^{Bd}	0.801 ± 0.04^{Cc}	1.113 ± 0.13^{Dd}	1.152 ± 0.03^{Dd}

Values are means ± standard error

C control (without Z-GE-A-MAP), *MAP* modified atmosphere packaging (without Z-GE-A), *Z* zein coating, *Z-GE 1.5%* zein with ginger extract 1.5%, *Z-GE 3%* zein with ginger extract 3%, *Z-GE 1.5%-A* zein with ginger extract 1.5% and AEO, *Z-GE 3%-A* zein with ginger extract 3% and AEO

Within each column means with the same lowercase letters are not significantly different ($P > 0.05$). The same uppercase letters are not significantly different between different experimental days for each treatment used ($P > 0.05$)

and Bazargani-Gilani [60] who revealed that chicken meat treated with apple peel extract and zein coating impregnated with ginger EO had lower TBA values compared to the uncoated samples during 12 days storage. The acceptable level of TBA for meat and meat products is recommended to be about 1 mg MDA/kg sample [48]. In the current study, the Z-GE 3%-A samples had approximately acceptable amount of TBA on day 20 of storage.

Sensory properties

In the present study, the 9-point hedonic scale was used for sensory evaluation of meat samples and the mean score over 7 (≥ 7) was regarded as a highly acceptable organoleptic feature. Alterations in organoleptic characteristics of treated meats during refrigerated storage are presented in Table 3. Overall, acceptability of sensory properties of samples in terms of color, odor, texture and general acceptance were markedly dropped during the storage period ($P < 0.05$). Based on the results of meat cooking on day 0, the addition of GE together with AEO partially affected the taste and reduced its score, while the addition of GE alone even at higher concentration was not significantly different from the control sample. This indicates the compatibility of the ginger flavor with the meat, which was considered desirable by the panelists. In terms of color parameter, the data showed that the addition of GE and AEO had a negative impact on the color of meat samples, while at the end of storage time, treated samples gained markedly higher scores compared to the uncoated (control) and Z treatments ($P < 0.05$). The odor assessment of uncoated and MAP only treated meats indicated unacceptable changes after 8 days of storage. In the case of odor parameter, treatment with GE and AEO gained lower and higher scores at the beginning and end of keeping time, respectively ($P < 0.05$). On the first day of the experiment, no significant difference was observed between the samples according to the texture scores ($P > 0.05$). Over time, the texture scores of all samples decreased significantly, which could be due to the breakdown of protein. Nevertheless, the rate of this reduction in GE and AEO treated meats was lower than other meat samples. The results of overall acceptance showed that the Z-GE 1.5%-A and Z-GE 3%-A treatments received satisfactory scores on day 16, while only Z-GE 3%-A treatment was acceptable to the panelists until the last day of storage (day 20). In the current study, uncoated (controls) and Z-GE 3%-A treated meats were the most perishable and efficient groups, respectively, which is probably due to the chemical and microbial alterations. It was found that GE-AEO combination effectively suppressed the lipid oxidation and microbial spoilage. Barkhordari and Bazargani-Gilani [60] revealed that Z coating had the positive impact on

Table 3 Alterations in sensory properties of MAP-treated beef samples during 20 days of refrigerated storage

Sensory attributes	Treatment	Storage time (day)					
		0	4	8	12	16	20
Taste	C	8.5 ± 0.62^{ab}	–	–	–	–	–
	MAP	8.3 ± 0.33^{b}					
	Z	8.6 ± 0.82^{ab}	–	–	–	–	–
	Z-GE 1.5%	8.6 ± 0.57^{ab}	–	–	–	–	–
	Z-GE 3%	9.0 ± 0.00^{a}	–	–	–	–	–
	Z-GE 1.5%-A	7.5 ± 0.24^{c}	–	–	–	–	–
	Z-GE 3%-A	7.9 ± 0.51^{c}	–	–	–	–	–
Color	C	8.9 ± 0.43^{a}	7.9 ± 0.00^{a}	5.5 ± 0.31^{c}	4.0 ± 0.00^{d}	1.1 ± 0.52^{e}	0.0 ± 0.00^{d}
	MAP	9.0 ± 0.00^{a}	8.0 ± 0.26^{a}	6.5 ± 0.85^{ab}	4.7 ± 0.42^{c}	1.7 ± 0.78^{d}	1.0 ± 0.00^{d}
	Z	8.7 ± 0.37^{a}	7.9 ± 0.82^{a}	6.5 ± 0.51^{ab}	5.6 ± 0.56^{b}	2.2 ± 0.57^{d}	1.2 ± 0.36^{d}
	Z-GE 1.5%	8.1 ± 0.41^{b}	7.2 ± 0.72^{b}	6.9 ± 0.73^{a}	5.8 ± 0.73^{a}	3.8 ± 0.48^{c}	2.7 ± 0.42^{c}
	Z-GE 3%	7.9 ± 0.38^{b}	7.5 ± 0.31^{b}	6.8 ± 0.45^{a}	5.4 ± 0.55^{b}	5.1 ± 0.34^{b}	3.4 ± 0.26^{b}
	Z-GE 1.5%-A	7.9 ± 0.59^{b}	7.3 ± 0.47^{b}	6.5 ± 0.22^{ab}	5.9 ± 0.62^{ab}	5.6 ± 0.62^{ab}	5.1 ± 0.35^{a}
	Z-GE 3%-A	7.8 ± 0.38^{b}	7.5 ± 0.00^{b}	6.4 ± 0.00^{b}	6.1 ± 0.23^{a}	6.0 ± 0.72^{a}	5.2 ± 0.87^{a}
Odor	C	8.8 ± 0.69^{a}	7.3 ± 0.34^{b}	5.7 ± 0.57^{c}	4.1 ± 0.42^{d}	1.3 ± 0.48^{d}	0.0 ± 0.00^{d}
	MAP	8.7 ± 0.34^{a}	7.9 ± 0.68^{ab}	5.6 ± 0.36^{c}	3.9 ± 0.46^{d}	1.3 ± 0.51^{d}	0.0 ± 0.00^{d}
	Z	8.7 ± 0.72^{a}	8.0 ± 0.00^{a}	7.1 ± 0.71^{b}	5.6 ± 0.38^{c}	4.0 ± 0.00^{c}	3.1 ± 0.42^{c}
	Z-GE 1.5%	8.6 ± 0.36^{a}	7.9 ± 0.44^{ab}	7.5 ± 0.66^{a}	6.4 ± 0.72^{b}	6.0 ± 0.63^{b}	6.0 ± 0.58^{b}
	Z-GE 3%	8.1 ± 0.83^{b}	8.1 ± 0.28^{a}	7.7 ± 0.89^{a}	6.7 ± 0.95^{b}	6.5 ± 0.38^{ab}	6.2 ± 0.21^{b}
	Z-GE 1.5%-A	8.1 ± 0.51^{b}	7.4 ± 0.43^{b}	7.2 ± 0.57^{a}	6.9 ± 0.68^{ab}	6.7 ± 0.64^{ab}	6.5 ± 0.46^{ab}
	Z-GE 3%-A	7.9 ± 0.48^{b}	7.6 ± 0.61^{b}	7.6 ± 0.36^{a}	7.4 ± 0.37^{a}	7.1 ± 0.47^{a}	7.0 ± 0.34^{a}
Texture	C	9.0 ± 0.00^{a}	8.1 ± 0.39^{b}	6.3 ± 0.83^{c}	4.1 ± 0.37^{d}	1.3 ± 0.39^{d}	0.0 ± 0.00^{g}
	MAP	9.0 ± 0.00^{a}	8.3 ± 0.74^{b}	6.6 ± 0.68^{c}	4.5 ± 0.21^{d}	1.5 ± 0.62^{d}	1.1 ± 0.38^{f}
	Z	8.8 ± 0.72^{a}	8.6 ± 0.51^{a}	8.2 ± 0.70^{a}	5.4 ± 0.89^{c}	1.3 ± 0.46^{d}	3.2 ± 0.71^{e}
	Z-GE 1.5%	8.7 ± 0.35^{a}	8.3 ± 0.48^{b}	8.1 ± 0.58^{ab}	8.0 ± 0.00^{a}	5.0 ± 0.00^{c}	4.1 ± 0.67^{d}
	Z-GE 3%	8.9 ± 0.44^{a}	8.4 ± 0.82^{ab}	7.7 ± 0.33^{b}	7.2 ± 0.36^{b}	5.6 ± 0.55^{b}	5.3 ± 0.29^{c}
	Z-GE 1.5%-A	8.8 ± 0.68^{a}	8.8 ± 0.59^{a}	7.8 ± 0.41^{b}	7.8 ± 0.48^{a}	7.7 ± 0.46^{a}	6.4 ± 0.56^{b}
	Z-GE 3%-A	9.0 ± 0.00^{a}	8.7 ± 0.46^{a}	8.3 ± 0.39^{a}	8.1 ± 0.57^{a}	7.7 ± 0.49^{a}	7.2 ± 0.34^{a}
	C	8.7 ± 0.53^{a}	5.8 ± 0.69^{c}	3.5 ± 0.37^{e}	1.4 ± 0.52^{f}	1.1 ± 0.78^{d}	1.1 ± 0.38^{f}
	MAP	8.8 ± 0.71^{a}	5.8 ± 0.41^{c}	3.7 ± 0.44^{e}	1.7 ± 0.46^{f}	1.2 ± 0.28^{d}	1.4 ± 0.42^{f}
	Z	8.0 ± 0.00^{b}	7.7 ± 0.39^{b}	5.3 ± 0.72^{d}	4.9 ± 0.38^{e}	4.6 ± 0.52^{c}	2.9 ± 0.33^{e}
Overall	Z-GE 1.5%	8.5 ± 0.56^{a}	8.4 ± 0.72^{a}	6.6 ± 0.52^{c}	6.2 ± 0.52^{d}	6.0 ± 0.00^{b}	4.4 ± 0.71^{d}
	Z-GE 3%	8.5 ± 0.82^{a}	8.6 ± 0.46^{a}	7.00 ± 0.00^{b}	6.6 ± 0.79^{c}	6.3 ± 0.31^{b}	5.7 ± 0.44^{c}
	Z-GE 1.5%-A	8.0 ± 0.35^{b}	8.2 ± 0.59^{ab}	7.4 ± 0.48^{ab}	7.1 ± 0.62^{b}	7.0 ± 0.23^{a}	6.4 ± 0.62^{b}
	Z-GE 3%-A	7.9 ± 0.54^{b}	7.8 ± 0.38^{b}	7.5 ± 0.72^{a}	7.4 ± 0.51^{a}	7.2 ± 0.75^{a}	7.0 ± 0.57^{a}

Values are means ± standard error

C control (without Z-GE-A-MAP), *MAP* modified atmosphere packaging (without Z-GE-A), *Z* zein coating, *Z-GE 1.5%* zein with ginger extract 1.5%, *Z-GE 3%* zein with ginger extract 3%, *Z-GE 1.5%-A* zein with ginger extract 1.5% and AEO, *Z-GE 3%-A* zein with ginger extract 3% and AEO

Within each column means with the same lowercase letters are not significantly different ($P > 0.05$)

the color features of chicken thigh meat. Similarly, it was indicated that enrichment of Z coating with plant extracts can improve the sensory properties of rainbow trout [21]. Previously, it has been shown that gas compositions containing $CO_2/O_2/N_2$:30/70/0 and $CO_2/O_2/N_2$:30/50/20 could efficiently maintain the acceptable red color of minced beef samples in modified atmosphere packs [39].

Conclusions

The findings of this study revealed that the application of zein coating enriched with GE and AEO for fresh beef packed in modified atmosphere led to shelf-life enhancement during storage at 4 °C. Moreover, these treatments showed acceptable sensory properties in fresh red meat. The findings

of this research indicated that combination of AEO and GE had more notable inhibitory impact on microbial spoilage and lipid oxidation than using GE alone. In addition, high oxygen concentration in MAP technique increased the lipid oxidation reactions while Z-GE-A treatment exhibited the ability to delay the chemical and microbial alterations. Accordingly, Z-GE 3%-A is proposed as the best treatment with the highest inhibitory impact which can maintain the fresh beef approximately up to 20 days under the refrigerated condition. However, further experiments are needed to assess the impact of Z-GE-A on other types of meat as well as other meat products with other kind of packaging methods.

Acknowledgements This research was financially supported by the Fasa University of Medical Sciences, Fasa, Iran and was carried out within the framework of the research project no. 99122.

Data availability Research data are not shared.

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