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Impact of an Edible Blend Coating Composed of Chitosan, Carrageenan, and Starch Enriched with *Berberis Vulgaris* Alcoholic Extract on Oxidative, Physicochemical, and Sensory Attributes of Hamburger Patties

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ABSTRACT

Background: Hamburger patties are susceptible to quality deterioration due to oxidative spoilage. The current research evaluated the effect of edible blend coating of chitosan/carrageenan/starch (EBC) enriched with *Berberis vulgaris* alcoholic extract (BE) on hamburger patties' oxidative, physicochemical, and sensory characteristics.

Methods: Anti-radical capacity of BE was determined by DPPH and H_2O_2 scavenging tests. Different EBC containing various concentrations of BE (1-3% *w/v*) was prepared. Weight loss (WL), pH, peroxide value (PV), thiobarbituric acid reactive substance (TBARS), and sensory characteristics of hamburger patties were evaluated during 16 days of storage at 4 °C.

Results: The BE's DPPH and H_2O_2 scavenging activities were 52.5% and 46%, respectively. The oxidative stability of hamburger patties was improved using bio-edible coating enriched with 2% w/v of BE (EBC/BE2). No significant differences were observed between the results of EBC/BE2 and EBC/BE3. Moreover, the implementation of EBC/BE2 prolonged the stability of the physicochemical and sensory characteristics of the patties during 16 days of cold storage at 4 ºC.

Conclusion: These results confirmed that BE is a valuable source of bioactive compounds possessing antiradical capacity. Furthermore, EBC/BE2 could be used as a coating material to decrease and postpone chemical and oxidative spoilage of hamburger patties by retarding moisture loss, reducing lipid oxidation, and eliminating dripping and discoloration.

1. Introduction

Meat-based products are very popular worldwide; however, they are particularly susceptible to discoloration, surface dehydration, and lipid oxidation. These factors can lead to health issues, financial losses, and diminished customer satisfaction (Latoch et al., 2023). Packaging could guarantee the quality and safety of meat and meat-based materials during handling, distribution, and storage. Environmental pollution from synthetic petroleum-based packaging materials has drawn consumers' and researchers' attention toward using natural, non-toxic, and environmentally friendly materials (Khin et al., 2024). In this regard, using edible coatings developed from natural sources is a promising option and a sustainable solution to solve ecological problems and ensure the preservation of the nutritional properties and quality characteristics of perishable foods (Mellinas et al., 2020). Carrageenan can be extracted from red seaweeds that belong to the *Rhodophyceae*. Carrageenan consisted of alternate units of 3, 6-anhydro-D-galactose, and D-galactose linked by α-1, 3, and β-1, 4-glycosidic bonds. Edible coatings containing carrageenan were used in various food industry sectors considering their significant and acceptable capacity of

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edible coating material. Some of the main limiting properties of their application are hydrophilicity, fragility, and low elasticity (Zhou et al., 2021). As a linear cationic polysaccharide, chitosan is an allergen-free, nontoxic, and biocompatible polymer. It was obtained by deacetylation in a concentrated alkali solution of chitin, which is observed in lobster, shrimp, and crab shells. Chitosan consists of N-glucosamine units and a small amount of N-acetyl glucosamine joined by beta linkages (1→4), producing resistant and flexible coatings with efficient carbon dioxide and oxygen barrier properties. Furthermore, chitosan is a biodegradable polymer possessing antioxidant properties (Hassan et al., 2018). Starch is a natural polymer consisting of anhydrous-glucose units containing amylopectin (branched-chain) and amylose (linear-chain). Edible starch coatings were used considerably, considering their tasteless, odorless, transparent, and biodegradable characteristics. This coating shows acceptable carbon dioxide and oxygen barrier properties. In this study, corn starch was used as an edible coating material. It is a white, odorless, and tasteless powder that is used in food processing. The primary limiting step of their application is their high hydrophilicity which is soluble in water and presents a weak water vapor barrier (Acosta et al., 2015; Dehghani et al., 2018a). Blend, composite, or multi-component coatings attempt to discover new complementary benefits of each component to minimize the limiting issues. Generally, blend coatings perform better than pure coatings, especially considering their mechanical, permeability, and moisture barriers. Blend coatings could be applied in multiple layers (bilayers), emulsions, or dispersions (Kurek et al., 2014). Moreover, the coatings could carry various additives such as antioxidants and antimicrobial agents to preserve desirable concentrations of target compounds on the surface of products. Synthetic antioxidants have been widely used to increase the shelf life of food products. However, there are some concerns about their possible toxicity and adverse impacts on human health (Tena & Asuero, 2020). In recent years, increasing awareness among consumers regarding synthetic additives and their demand for high-quality products has created opportunities for exploring new, sustainable natural ingredients derived from plant sources. Bioactive compounds include secondary compounds from plant metabolism, such as vitamins, enzymes, and phenolic compounds. Phenolic compounds are common natural antimicrobial and antioxidant agents, including flavonoids, phenolic acids, tannins, etc. (Pasquet et al., 2024). According to different studies, antioxidant compounds are added to food products to delay or prevent the initiation or propagation of oxidation reactions (Heydari et al., 2020; Samani et al., 2022). The *Berberis vulgaris* belongs to the species of Berberidaceae family and is native to the mountainous regions of the Mediterranean in Asia and Europe and is notably found in Iran's South Khorasan Province. In the traditional medicine of Iran, the root, stem, leaf, flower, and fruit of *B. vulgaris* are used due to their antioxidants, antibacterial, anti-tumor, and anti-fever properties (Mueed et al., 2024; Behravan et al., 2019). Bioactive compounds from different natural sources are essential to human health (Fazeli & Bimakr, 2024). Edible coatings attract scientists' interest as they are biodegradable, non-toxic, cheap, and available. Furthermore, they can maintain the nutritional value of products. They may also improve stability and control the release of bioactive compounds during extended periods. For this purpose, natural compounds obtained from sources such as pomegranate peel (Alsaggaf et al., 2017), green tea (Özvural et al., 2016), *Satureja hortensis* (Farahani et al., 2024), *Zataria multiflora* (Mehdizadeh & Langroodi, 2019), dill essential oil (Shahedi et al., 2024), and so on have been incorporated into edible coatings to delay the deterioration of the meat-based products during storage significantly. Therefore, the current study investigated the effect of a blended edible coating of starch-carrageenan-chitosan containing *B. vulgaris* alcoholic extract on the physicochemical (pH and WL) and sensory qualities (color, odor, texture, and overall acceptability) and also oxidative spoilage (PV and TBARS) of hamburger patties during 16 days of storage at refrigeration.

2. Materials and Methods

2.1 Materials

Dried *B. vulgaris* aerial parts were obtained from Zanjan, Iran. Then, the sample was grounded into fine particles using a grinder (GSC-911, Gosonic, China) and kept for further experiments at 4 °C. Veal meat was acquired from a local market in Zanjan, Iran. Corn starch (Golha, Tehran, Iran) was purchased from a local market. Ethanol, methanol, hydrogen peroxide (30%), and hexane were purchased from Dr. Mojallali Co. (Markazi, Iran). Other chemicals were obtained from Sigma-Aldrich, St. Louis, MO, USA.

2.2 Preparation of B. vulgaris alcoholic extract

Extraction processes were carried out using a thermo-sonication procedure. Briefly, 5 g of sample powder was added to 150 mL of proper solvent (ethanol 70% *v/v*) and maintained for 5 h at ambient temperature (23 ± 1 °C). Then, the extraction was performed using an ultrasonic bath (vCLEAN 1-L9, Backer Viera Trading, Tehran, Iran) at 50 °C for 1 h. The extraction procedure was completed by stirring the mixture for 3 h at 50 °C using a magnetic stirrer (HMS 8805, Ghatran Shimi Tajhiz, Tehran, Iran). The mixture was filtered through filter paper, and the solvent was removed using a rotary vacuum evaporator (R-250, Flawil, Switzerland) at 40 °C. The resulting extract was stored in dark vials at -18 °C for further analysis (Bimakr et al., 2017).

2.3 Free radical scavenging analysis of B. vulgaris alcoholic extracts

2.3.1. 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) method

The DPPH radical scavenging capacity of the *B. vulgaris* alcoholic extract was measured based on the procedure previously described by Bimakr et al. (2017) with slight changes. Briefly, 0.1 mL of extract was added to 2.9 mL of

ethanol solution of DPPH (0.1 mM). After 30 min retention in the dark room, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (SPECORD 250, Analytik Jena, Thuringia, Germany). The scavenging activity of DPPH free radicals was calculated using Equation (1):

$$Scavenging\ activity\ of\ DPPH\ (\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100 \qquad \text{(Eq. 1)}$$

2.3.2 Hydrogen peroxide (H_2O_2) method

The H_2O_2 scavenging capacity of the *B. vulgaris* extract was measured based on the Boulekbache-Makhlouf et al. (2013) method with some changes. Briefly, 1 mL of the extract was mixed with a specific amount of 30% of the H_2O_2 solution. Absorbance was read at 530 nm by a UV-Vis spectrophotometer. The scavenging activity of hydroxyl radical was calculated using Equation (2):

$$Scavenging\ activity\ of\ H2O2(\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100 \qquad \text{(Eq. 2)}$$

2.4 Preparation of chitosan/carrageenan/starch coatings

To prepare the edible blend coating of chitosan/carrageenan/starch (EBC) emulsion, 1 g of chitosan, carrageenan, and starch was dissolved separately in 100 mL of deionized water. The glycerol (25% *w/w*) was mixed with EBC emulsion and stirred for 30 min using a magnetic stirrer. *B. vulgaris* extract (BE) was added at three different concentrations of 1, 2, and 3% *w/v* to prepare the bio-edible coating. Tween 80 was added as an emulsifier, and then the emulsion was thoroughly mixed for 30 min with a magnetic stirrer. Finally, the sample was homogenized using a homogenizer (SR 30, MTOPS, Seoul, South Korea) at 15000 rpm for 8 min (Zhou et al., 2021).

2.5 Preparation of hamburger patties and coating process

Veal meat was directly transferred within 1 h in an insulated ice box to the laboratory. Different ingredients, such as onion, flour, garlic, and salt, were mixed with minced meat, and samples (150-160 g, 1 cm thickness) were prepared manually using a steel mold. Finally, prepared samples were immersed in coating emulsion for 1 min. The samples were allowed to drain for 1 min, and the process was duplicated. Finally, the coated samples were wrapped gently in polyethylene bags and kept in a refrigerator (Roshani Neshat et al., 2022). The physicochemical and sensory analyses were performed at 4-day intervals during 16 days of refrigerated storage (4 °C). Five treatments were prepared and analyzed including control 1 (C1, non-coated hamburger patties); control 2 (C2, coated hamburger patties with EBC without *B. vulgaris* extract); EBC/BE1 (coated hamburger patties incorporated with 1% *w/v B. vulgaris* extract in EBC), EBC/BE2 (coated hamburger patties incorporated with 2% *w/v B. vulgaris* extract in EBC), and EBC/BE3 (coated hamburger patties incorporated with 3% *w/v B. vulgaris* extract in EBC).

2.6 Lipid oxidation

The peroxide value (PV) and thiobarbituric acid-reactive substance (TBARS) were measured to evaluate the lipid oxidation of the treated samples during 16 days of cold storage.

2.6.1 Peroxide value

To measure the peroxide value (PV), 0.5 g of sample was mixed with 25 mL of glacial acetic acid-chloroform solution. After adding KI (1 mL), it was kept in a dark place for 10 min. Distilled water (30 mL) and starch indicator solution (1 mL) were added to the sample. After mixing, the mixture was titrated with 0.01 N $K_2S_2O_3$, and the results were expressed as milliequivalents (meq) of oxygen kg^{-1} using Equation 3 (Joukar et al., 2017).

$$\text{PV (meq } O_2\text{/kg)} = \frac{\text{(titration volume of sample (mL)} - \text{titration volume of blank (mL))} \times \text{K2S2O3 concentration (mol/L)} \times 1000}{\text{sample weight (g)}} \qquad \text{(Eq. 3)}$$

2.6.2 Thiobarbituric acid value

The values of thiobarbituric acid-reactive substance (TBARS) were determined based on colorimetry using the Dehghani et al. (2018b) method. For this purpose, 200 mg of the homogenized sample was added to 25 mL of 1-butanol. Then, 5 mL of this mixture was combined with 5 mL of a fresh thiobarbituric reagent solution. The test tube was placed in a hot water bath for 90 min and then cooled in an ice water bath for 10 min. The absorbance of the sample (A_s) was measured using a UV-Vis spectrophotometer at a wavelength of 532 nm. A blank sample (A_b) was prepared similarly, and its absorbance was also determined. The MDA standard curve was also prepared. The TBARS value was calculated and expressed as mg of malondialdehyde per kg of the sample according to Equation 4 (Roshani Neshat et al., 2022).

$$TBARS = 50 \times \left(\frac{A_s - A_b}{200}\right) \qquad \text{(Eq. 4)}$$

2.7 Measurement of weight loss

To determine the weight loss (WL) of the samples, the initial weight (W_0) and weight (W_1) of samples during 16 days of cold storage for each treatment were recorded, and the WL was calculated using Equation 5 (Zhou et al., 2021).

$$WL\ (\%) = \frac{W_0 - W_1}{W_0} \times 100 \qquad \text{(Eq. 5)}$$

2.8 Measurement of pH

The pH values of samples were measured according to Saricaoglu and Turhan (2019). A total of 5 g of the sample was mixed with distilled water in a 1:10 ratio. The mixture was homogenized with a homogenizer at 8000 rpm for 2 min. Finally, the pH was measured using a digital pH meter (AZ86502, AZ Instrument, Taichung, Taiwan).

2.9 Evaluation of sensory properties

20 semi-trained graduate students (10 males and 10 females aged between 20 and 26) carried out the sensorial evaluation of samples. The five-point hedonic scale (1: really dislike, 5: really like) was used for the sensory evaluation of samples regarding color, odor, texture, and overall acceptability. All samples were coded and offered to the panelists in random order (Zhou et al., 2021).

2.10 Statistical analysis

All experiments were conducted in triplicate, and the experimental data were expressed as mean ± standard deviation (SD). The means were separated by one-way analysis of variance (ANOVA) applying the Tukey test at a 95% confidence level ($p \leq 0.05$) using Minitab software version 16.02.04 (State College, PA, USA).

3. Results and Discussion

3.1 Free radical scavenging ability of B. vulgaris alcoholic extract

The current study determined the free radical scavenging ability (FRSA) of *B. vulgaris* extracts using DPPH and H_2O_2 assays. According to the results, *B. vulgaris* extract possessed considerable FRSA (%$DPPH_{sc}$ of 52.50 ± 0.22% and %HO_{sc} of 46.00 ± 0.16%). From the results, the higher radical scavenging activity was obtained in the DPPH assay, while the lower scavenging activity was obtained in the H_2O_2 assay ($p < 0.05$). The same behavior was reported by Mojarradi et al. (2021). They stated that the hydroethanolic solvent system (HSS) was efficient for the recovery of bioactive compounds from *Silybum marianum* areal parts. They reported *S. marianum* alcoholic extract could be considered a natural ingredient for the food and pharmaceutical industries since it possesses acceptable antiradical capacity (39.19 ± 0.30 % $DPPH_{sc}$ and 28.15 ± 0.51 % OH_{sc}). The results show that *B. vulgaris* alcoholic extract is a potential source of antiradical scavengers, highlighting its importance for further application in the food industry. However, applying other novel extraction techniques, such as microwave-assisted extraction and supercritical carbon dioxide extraction, may lead to obtaining extracts with higher FRSA. It should be remembered that free radicals are unstable atoms with the ability to attack intact cells. They are linked to aging and a diverse group of diseases. Furthermore, free radicals significantly accelerate lipid oxidation in various food products. Therefore, determining the FRSA of extracts is necessary to develop products that improve consumer health and postpone product spoilage (Blinova et al., 2024; Daliri Sosefi et al., 2024). Several articles reported that natural bioactive compounds may positively affect human health, postpone product spoilage, and prolong the product shelf-life (Mojarradi et al., 2024; Roshani Neshat et al., 2022). According to the current findings, *B. vulgaris* is a valuable source of bioactive compounds containing considerable FRSA, emphasizing the significance of its application in producing bio-edible coatings.

3.2 Application of EBC coating containing B. vulgaris extract on hamburger patties

3.2.1 Weight loss changes

Weight loss (WL) changes in hamburger patties were observed after applying different bio-coating treatments during 16 days of cold storage, as shown in Figure 1. Fat loss and water loss during cold storage of products are major reasons for WL. This phenomenon had an important effect on the final appearance of meat products. From the results, the WL revealed an increasing trend during storage; however, the type of treatment had a considerable effect on the increasing trend. The highest WL value was found in the C1 sample at the end of storage (20.43 ± 0.16%). In contrast, the lowest value of WL was observed in samples treated with EBC-BE, particularly EBC/BE2 (12.00 ± 0.30%) and EBC/BE3 (11.80 ± 0.35%), which had a significant difference with C1 ($p < 0.05$). It should be noted that the WL measurement of C1 on the 16th day of storage was not performed due to the severe spoiled conditions of the sample after 12 days. Meat loses weight through surface evaporation. This process depends on temperature and relative humidity differences between the meat and the environment (Kalem et al., 2018). The results are consistent with those reported by Vital et al. (2016). Edible coatings can be a barrier to gases and water vapor, inhibiting moisture and, hence, weight loss during product storage (Kalem et al., 2018). Furthermore, the appropriate amount of BE can be evenly distributed within the EBC without compromising the protective layer's functions. This distribution plays a vital role in proper coating formation, helping to prevent water and fat loss. In addition, moisture loss from foods was slowed by polysaccharide-based coatings, as these coatings acted as sacrificing agents. Application of EBC-BE eliminated dripping loss of samples during cold storage preserving the product quality and appearance. The result was consistent with Zhou et al. (2021) and Kalem et al. (2018).

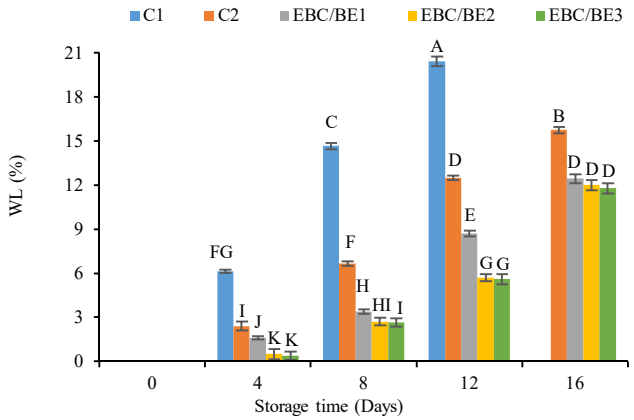

Figure 1. Effect of different treatments on WL changes of hamburger patties during cold storage. Numbers with similar capital letters do not differ significantly ($p > 0.05$)

3.3 Changes of pH

Changes in the pH of hamburger patties applying different treatments of bio-coating during 16 days of cold storage (4 ºC) are represented in Figure 2. The pH level of meat is a crucial factor that affects its freshness, flavor, and overall quality. Thus, measuring the pH of meat is essential for maintaining its quality (Roshani Neshat et al., 2022). The initial pH values ranged from 5.55 to 6.00. These results aligned with those of a different study (Lashkari et al., 2020). However, various parameters such as season, diet, species, and stress levels before and during slaughtering affect the pH values of meats (Mojarradi et al., 2024; Roshani Neshat et al., 2022). According to the results, applying EBC-BE considerably affected the pH changes of samples. Considering Figure 2, an increasing trend was evident with increasing storage time in all samples with different rates depending on the type of treatment. The highest increment was observed in the control sample (C1). It should be noted that the pH measurement of C1 on the 16th day of storage was not performed due to the severely spoiled conditions of the sample after 12 days. Generally, pH increment during storage might be attributed to bacterial activity in the early stage of storage, which produces different protein metabolites such as ammonia and amines (Roshani Neshat et al., 2022). From the results, the pH value of C2 is lower than C1, which suggests the positive controlling effect of EBC on pH changes. It should be stated that the pH values of the samples treated with EBC-BE were lower than control samples (C1 and C2). A moderate pH increment was observed by adding *B. vulgaris* extract, particularly at 2 and 3% v/w concentrations. There is no significant difference between EBC/BE2 and EBC/BE3 results (p > 0.05), which suggests the adequacy of 2% BE. The lower rate of pH increment applying EBC-BE might be due to the antioxidant activity of bioactive compounds that exist in *B. vulgaris* extracts, such as phenolic compounds, which scavenge free radicals and retard the oxidation process, resulting in lower pH values (Mojarradi et al., 2024). Comparable results were also reported by Zhou et al. (2021) and Lashkari et al. (2020).

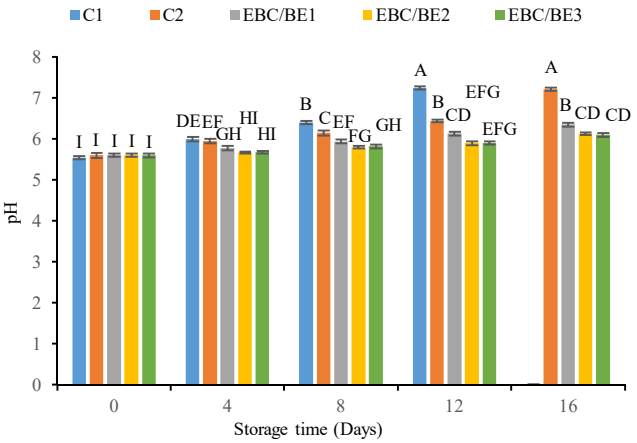

Figure 2. Effect of different treatments on pH changes of hamburger patties during cold storage. Numbers with similar capital letters do not differ significantly (p > 0.05)

3.4 Changes of PV

The effects of the different treatments on PV changes of hamburgers are depicted in Figure 3. Different active agents, such as temperature, light, oxygen, and chemical oxidants, produce free radicals from fatty acids, leading to lipid oxidation products. This phenomenon resulted in adverse effects on the nutritional and physicochemical properties of the hamburgers during storage, such as off-flavor and discoloration (Bazargani-Gilani et al., 2015). One of the major assessments to estimate the lipid oxidation of products during storage is PV measurements. This value indicates the quantity of primary products resulting from lipid oxidation, particularly hydroperoxides. In another study, Alizadeh Behbahani et al. (2021) reported that the maximum allowable level of hydroperoxides in veal meat is 7 meq peroxide kg^{-1}. In the current study, it was found that C1 exceeded that value after 8 days of cold storage, while EBC/BE3 and EBC/BE2 were lower than this value at the end of storage.

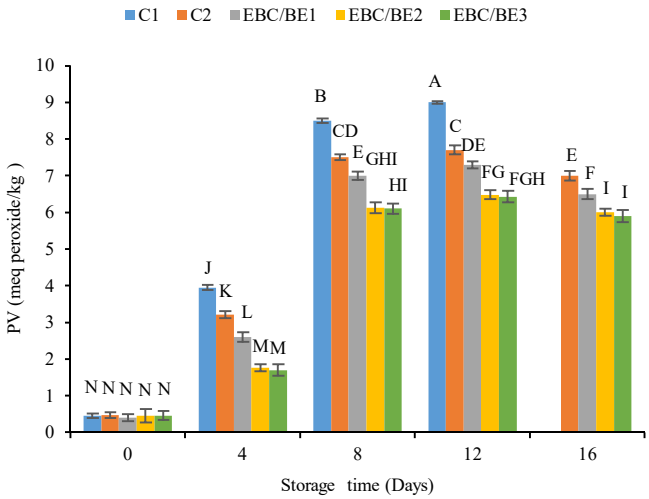

Figure 3. Effect of different treatments on PV changes of hamburger patties during cold storage. Numbers with similar capital letters do not differ significantly (p > 0.05)

It should be mentioned that the C1 was analyzed during 12 days of storage, and after that, the sample was highly spoiled. The results indicated a clear ascending trend at varying rates for all samples during cold storage. However, the type of treatment had a significant effect on PV (p < 0.05). Among the samples, the PV value of EBC/BE3 showed the lowest value (5.90 meq active oxygen kg^{-1}); however, no significant difference existed between the samples related to EBC/BE2 (p > 0.05). In contrast, the highest value of PV was found in C1. These results reflected the inhibitory effect of EBC-BE on the lipid oxidation of hamburgers during cold storage. The increases in PVs continued until day 8, and after that, gradually, a decreasing trend was observed until the end of storage. The decrease in PV is related to the decomposition of hydroperoxides, which resulted in the release of secondary oxidation products. Adding *B. vulgaris* extracts to the emulsion of edible coating reduced hydroperoxide

decomposition due to its radical scavenging activity, which was discussed earlier. Furthermore, Mojarradi et al. (2024) investigated the impact of a bio-edible coating enriched with bioactive compounds from *Malva sylvestris* leaves on prolonging the shelf-life of turkey meat. They stated that the enrichment of bio-edible coating with bioactive compounds had a remarkable inhibitory effect on the PV of the samples during cold storage. In our study, similar effects of bioactive compounds were found. The results from the characterization study of *B. vulgaris* extract confirmed these findings.

3.5 Changes of TBARS

Aldehydes and ketones are the main secondary lipid oxidation products, leading to product deterioration during storage. The concentration of these products could be measured using thiobarbituric acid reactive species (TBARS). The effects of the different treatments on TBARS changes in hamburger patties were depicted in Figure 4. From the results, on the first day of storage, the TBARS values ranged between 0.45 to 0.50 mg MDA kg^{-1}. It should be mentioned that the C1 was analyzed during 12 days of storage, and after that, the sample was highly spoiled. An upward trend of TBARS was observed in all samples. It was observed that applying the edible coating to the hamburger patties had a considerable effect on TBARS changes during cold storage. However, the TBARS value for C1 was significantly higher than those of the other treatments during storage ($p < 0.05$), which is in agreement with those reported by Jouki et al. (2020). It was reported that the maximum limit of TBARS value indicating good quality meat is 1 mg MDA kg^{-1} of tissue (Alizadeh Behbahani et al., 2021). In the current study, the TBARS of C1 were more than that threshold after twelve days. While TBARS of EBC/BE3 (0.64 ± 0.06 MDA Kg^{-1}) and EBC/BE2 (0.65 ± 0.07 MDA Kg^{-1}) were lower, which reflected the positive effect of bio-edible coating to control TBARS changes during cold storage (4 °C). According to the current results, bio-edible coating inhibited the production of secondary lipid oxidation products in hamburger patties. The increment of TBARS during storage is related to decomposing primary lipid oxidation products into secondary products such as aldehydes and ketones (Mojarradi et al., 2024). In our study, it was found that with decreasing the PV, an increasing trend was observed for TBARS values, which confirmed the conversion of primary products into secondary products. The combined effect of the edible coating and *B. vulgaris* bioactive compounds effectively inhibited changes in TBARS during the cold storage of the samples. The edible coating acts as a physical barrier, and *B. vulgaris* bioactive compounds possess considerable anti-radical activity, which prevents the production of secondary metabolites and, hence, TBARS changes. By comparing the TBARS results of C1 and C2, it was found that the development of secondary lipid oxidation products in C1 was higher than those related to C2. These findings show that coatings can inhibit lipid oxidation by restricting oxygen diffusion through the coating layer. Similar findings were reported by Roshani et al. (2022). The highest value of TBARS at the end of storage was obtained from C1, and the minimum TBARS belonged to EBC/BE3 (1.23 ± 0.06 MDA Kg^{-1}), which reflected the synergistic effect of edible coating and *B. vulgaris* bioactive compounds. The radical scavenging capacity of *B. vulgaris* extract considerably affected the delay of oxidative spoilage. This statement is supported by the evidence showing that increasing the concentration of bioactive compounds in bio-edible coatings positively impacts the control of lipid oxidation. These findings align with the research conducted by Panahi & Mohsenzadeh (2022) and Zou et al. (2022). Moreover, using an edible coating with green tea extract reduced the TBARS values of beef patties (Banon et al., 2007; 30. Özvural et al., 2016).

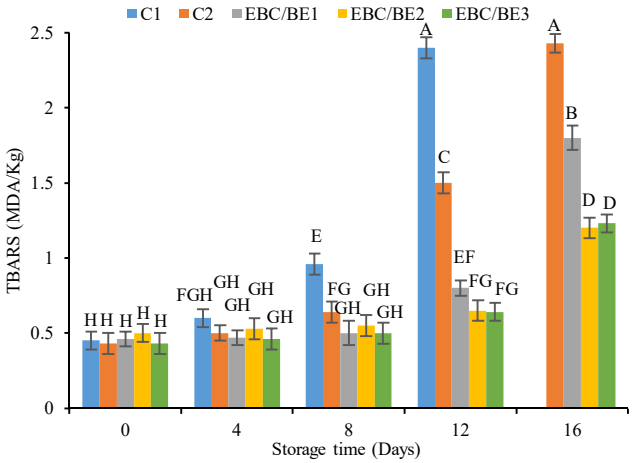

Figure 4. Effect of different treatments on TBARS changes of hamburger patties during cold storage. Numbers with similar capital letters do not differ significantly ($p > 0.05$)

3.6 Changes in sensory attributes

The changes in sensory attributes were used for evaluating the hamburger quality during cold storage, and the results are presented in Figure 5. In the current study, the scores of all sensory properties, including texture, odor, color, and overall acceptance, decreased during storage with different rates. Higher scores were obtained for the samples coated with edible coating containing *B. vulgrais* extract, particularly EBC/BE2 and EBC/BE3. The samples with scores lower than 3 were considered unacceptable, considering signs of off-flavor production and slimy appearance. On the initial day of storage, all samples achieved their highest scores. Subsequently, a downward trend was observed. In general, reducing the scores of sensory characteristics in meat and meat products during the storage period is considered a natural occurrence. A sharp decrease in all properties was observed on the 12th day of the storage. This could be due to the production of aldehydes, ketones, hydrocarbons, alcohols, and esters as secondary products of lipid oxidation (Shahimoridi et al., 2024). The lowest scores were detected in the C1 sample, reflecting the desirable effect of applying the edible coating to delay lipid oxidation. Furthermore, the enrichment of edible coating using *B.*

vulgaris bioactive compounds positively affected the score of sensorial attributes, revealing the efficiency of bioactive compounds in attenuating the lipid oxidation of coated hamburgers. From the 4th to the 8th day of storage, signs of spoilage were detected, especially in sample C1 ((odor (score of 1), color (score of 2.5), texture (score of 1), and overall acceptance (score of 2.1)), and this sample became unacceptable in terms of overall acceptance after 8 days of cold storage. This result can be due to the spread of chemical spoilage caused by the oxidation of lipids, which led to unpleasant color and smell and slimy texture in the samples (Samani et al., 2022). Based on the results, using an edible coating of EBC/BE2, hamburger patties can be stored for 12 days at refrigerator temperature without causing considerable adverse effects on sensory characteristics (odor (score of 3.5), color (score of 5), texture (score of 3.5), and overall acceptance (score of 3.6)). The results were consistent with those that enhance the sensory attributes of meat products coated with various bio-coatings (Shahimoridi et al., 2024; Barkhordari & Bazargani-Gilani, 2021).

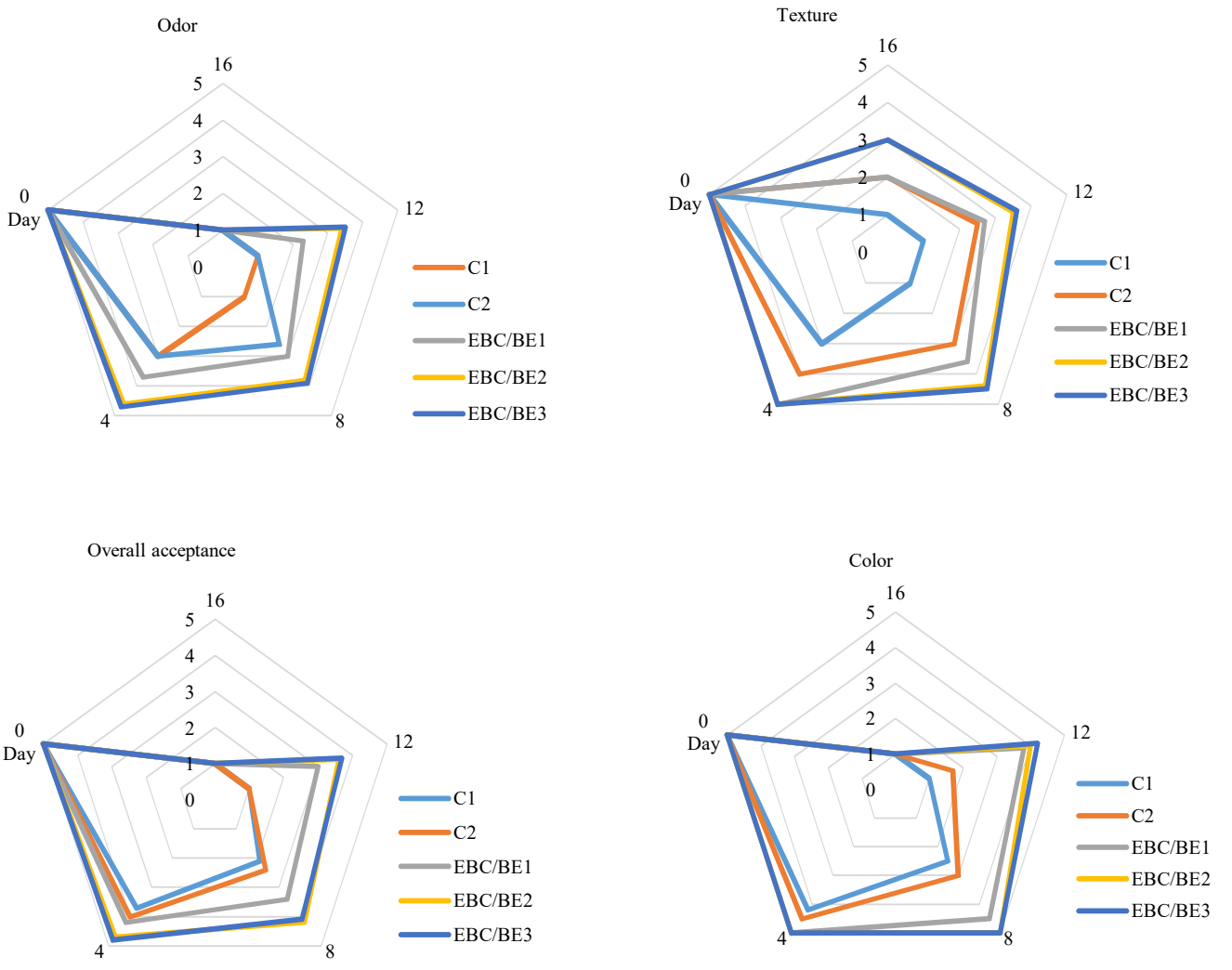

Figure 5. Effect of different treatments on changes of sensory attributes of hamburger patties during cold storage

4. Conclusion

The results presented in this study indicated that *B. vulgaris* extract is a potential source of natural bioactive compounds with significant free radical scavenging activity. Incorporating *B. vulgaris* extract into a natural blend coating of chitosan-carrageenan-starch fabricated an edible bioactive coating for possible meat product packaging applications. In this study, the oxidative stability of hamburger patties coated with a bio-edible coating based on chitosan-carrageenan-starch enriched with *B. vulgaris* extract was maximized for 12 days of cold storage of

hamburgers without any considerable negative effect on sensorial properties of odor, color, texture, and overall acceptance. After twelve days, the sensory attributes became unacceptable considering the oxidation of lipids, which led to unpleasant color and smell and slimy texture. Furthermore, the PV, TBARS, pH, and WL analyses confirmed these findings. This study suggests that a combination of *B. vulgaris* extract and chitosan-carrageenan-starch could be applied as a new packaging material to retard chemical and oxidative spoilage of hamburger patties during cold storage (4 ºC).

Authors' Contributions

Mehdi Kazemi: Literature review; Formal analysis; Investigation; Writing-original draft. **Mandana Bimakr:** Conceptualization; Formal analysis; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing. **Peymaneh Ghasemi Afshar:** Conceptualization; Formal analysis; Methodology; Writing-original draft.

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Conflicts of Interest

The Authors declare that there is no conflict of interest.

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Ethical considerations

No ethical considerations were considered in this research under Project number 162782459.

Using artificial intelligence

We hereby declare that artificial intelligence was not used in the current manuscript.

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