

Potentials of beetroot (*Beta vulgaris* L.) peel extract for quality enhancement of refrigerated beef meat

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Abstract

Given the significant quantity of betalain pigments, beetroot represents a potential source of natural colorants that can be employed in the food industry. The present investigation explored the impact of ethanolic beetroot peel (EBP) extract in beef meat preservation. EBP displayed a 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect with an IC_{50} value of 0.96 mg/mL. The anti-*S. aureus*, anti-*L. monocytogenes*, anti-*E. coli*, and anti-*Salmonella enterica* activity was assessed, and the minimum inhibitory concentration was ≤ 1.5 mg/mL. EBP extracts at three concentrations of 0.075%, 0.15%, and 0.3%, and butylated hydroxytoluene (BHT) concentration of 0.01%, employed at its recommended limit, were incorporated in refrigerated raw minced beef meat. The impact of these treatments on chemical stability, instrumental color, microbiological and sensory attributes of meat was monitored for 14 days at 4°C. At different levels, EBP extract led to a decrease in lipid/protein oxidation parameters and delayed microbial load throughout storage, with improved instrumental color and sensory traits. Interestingly, EBP extract at 0.3% has the strongest preservative effect until the end of storage. Using principal component analysis, effective discrimination was elucidated by linking sensory traits with chemical oxidation behavior, microbial alterations, and instrumental color. This investigation proved that EBP could be an encouraging natural additive in the meat industry.

Keywords: beetroot peel extract; biological activities; minced beef meat; quality evaluation; chemometric evaluation; shelf-life extension

Introduction

According to Food and Agriculture Organization (FAO, 2022) statistics, in 2021, the production of collective red meat in Africa was around 11 million tons. In Tunisia, the production was recorded at approximately 127 thousand tons (Agridata, 2021). It should be noted that 86% of this production was from bovines and ovines (Agridata, 2021).

In the meat industry, mitigating spoilage and oxidation is one of the most challenging tasks, as 20% of the total produced meat is spoiled annually (Awad *et al.*, 2022). For this purpose, searching for a suitable preservative method for raw meat is recommended. For instance, some studies conducted in Tunisia focused on preserving meat and meat derivatives. By using organic acids and their salts, Smaoui *et al.* (2011, 2012) and Chabbouh *et al.* (2012) reported that lactic acid and sodium lactate could be efficiently used in extending the shelf life of poultry thigh and kaddid, traditional salt meat. Moreover, Tunisian researchers applied endemic plants, viz. extracts and essential oils, for the preservation of meat. For this purpose, Fourati *et al.* (2020) and Mhalla *et al.* (2017) evaluated the potential of pomegranate peel extract and *Rumex tingitanus* extract to improve the quality of beef meat. In the studies conducted by Ben Akacha *et al.* (2022) and Smaoui *et al.* (2016), *Lobularia maritima* and *Mentha piperita* essential oils were efficiently used by delaying the lipid/protein oxidation process and enhancing sensory features. Moreover, polysaccharides, sourced from entities, such as algae or fungi, are currently garnering attention as natural preservatives. Eljoudi *et al.* (2022) and Hamzaoui *et al.* (2020) explored the utilization of polysaccharides for meat preservation. In another way, microorganisms and their metabolites as bacteriocins have displayed notable potential in beef meat. Chakchouk-Mtibaa *et al.* (2017) investigated the useful impact of bacteriocin (BacFL31) for preserving raw ground turkey meat.

Nowadays, consumers request healthy, safe, and vivid colors in food products. Synthetic colorant additives, such as Allura red (E129) (Siddiquee and Shafwanah, 2020) and ponceau 4R (E124) (Leulescu *et al.*, 2019), were widely utilized to enhance the shelf life and color of the product. However, consumption of these chemicals leads to dangerous health effects, such as hyperactivity and allergic reactions (Ribeiro *et al.*, 2019). The tendency to consume natural pigment additives has increased in light of this alarming situation. Consequently, a growing trend is observed in the food industry globally to substitute chemical colorants with natural additives.

Natural colorant preservatives can have a double-edged purpose by providing color and antioxidant properties. These additives were acquired from various plants to

extract antioxidants and pigments. Principal plant pigments comprised chlorophyll (green), anthocyanins (blue–red), carotenoids (yellow–orange), and betalains (red–purple–yellow) (Novais *et al.*, 2022; Zhang *et al.*, 2022). In this course, numerous studies investigated the potential of plant extracts, such as maqui (*Aristotelia chilensis*) (Velázquez *et al.*, 2022), red pitaya (Bellucci *et al.*, 2021), *Bixa Orellana* (Bolognesi and Garcia, 2018), red prickly pear pulp (Kharrat *et al.*, 2018; Palmeri *et al.*, 2018), and jabuticaba (*Myrciaria cauliflora*) (Baldin *et al.*, 2018; Tayebbeh *et al.*, 2021), for meat and meat products. It must be noted that color is the principal sensory quality influencing a food product's initial perception and market success (Manzoor *et al.*, 2021). Colors enhance food product's intensity, making it healthier and more appealing (Echegaray *et al.*, 2023). Nevertheless, natural additives are usually more expensive than synthetic ones. As a result, many studies focused on extracting antioxidants and/or colorants from low-cost materials, such as residual sources from agricultural industries. In this vein, beetroot peels, including phenolic compounds, such as phenolic acids and flavonoids, as well as betalains, are recognized as a valuable and abundant source of bioactive compounds and pigments (Chaari *et al.*, 2022b; Maqbool *et al.*, 2021), and are assigned as safe and beneficial for human consumption (Wang *et al.*, 2023).

Several investigations explored the meat quality features using a few parameters and manipulations, but the corresponding data were not linked properly. In order to place a large number of parameters, chemometrics, such as the application of principal component analysis (PCA) in conjunction with mathematical models, were utilized to assess meat quality (Muzolf-Panek *et al.*, 2020). These chemometrics involved the analysis of multiple variables and the interconnection of all quality parameters (Kharbach *et al.*, 2023). These also reduced data dimensionality while retaining the discriminating power (Wang *et al.*, 2014), and simultaneously offered rapid, sample, and cost-effective means of food analysis (Gonçalves *et al.*, 2021).

In this study, we attempted to (i) investigate the microbiological, oxidative stability, and instrumental color changes in beef meat samples treated with ethanolic beetroot peel (EBP) extract at different storage times, and (ii) link all corresponding data via PCA to better elucidate interconnections between quality parameters.

Materials and Methods

Ethanolic beetroot peel extract preparation

Red beetroot (*Beta vulgaris* L.) was obtained from a market in Sfax, Tunisia. The beetroot peels were subjected

to cleaning, peeling, and drying until they attained a stable weight. This drying procedure was done using a hot air oven set at 40°C. Subsequently, the dried beetroot peels were finely crushed to a powder and sifted to prepare a substrate for the extraction of betalains.

In order to prepare EBP, the optimum conditions of the Chaari *et al.* (2022a) study were used. These conditions included a sample-to-solvent ratio of 1:36 (red beetroot powder–ethanol, w/v), a temperature of 44.54°C, and an extraction time of 93.03 min.

Preparation of raw minced beef meat

Raw beef meat is obtained from a local Sfax (Tunisia) market. Then the samples were minced in a meat grinder. Concerning the conditioning of raw minced beef, five equal portions in the form of control, butylated hydroxytoluene (BHT)-treated, and 1-EBP, 2-EBP, and 4-EBP extract-treated samples were placed in separate sterile plastic bags. Four meat portions were mixed separately with BHT (0.01%) and EBP extracts (0.075% extract, 1-EBP), (0.15% extract, 2-EBP), and (0.3% extract, 4-EBP); 1-EBP, 2-EBP, and 4-EBP demonstrated a minimum inhibitory concentration (MIC) of 1×, 2×, and 4×, respectively. All meat samples were stored at 4°C and assessed at 0, 3, 7, 10, and 14 days.

Ethanollic beetroot peel extract analysis

Betalains analysis

The concentrations of betacyanins (Bc, red–violet) and betaxanthins (Bx, yellow) were assessed using a spectrophotometric method at 480 nm and 538 nm, respectively. Betacyanins and betaxanthins were utilized to quantify the total concentration of betalains (Bt) in EBP extract, as established by Righi Pessoa da Silva *et al.* (2018).

Total phenolic content (TPC) and total flavonoid content (TFC)

Total phenolic content was measured using the method described by Liang and Wang (2018). EBP extract at 100 µL was mixed with 400 µL of Folin–Ciocalteu reagent at 0.2 N. Then 7.5% sodium carbonate (Na₂CO₃, 500 µL) was added. The absorbance was determined at 765 nm after 2-h incubation. TPC was expressed as mg GA equivalents/g (mg GAE/g).

Total flavonoid content was quantified using the assay described by Saharan *et al.* (2020). EBP extract, 1 mL, was added to 1 mL of aluminum chloride (AlCl₃, 2%) and incubated for 1 h at room temperature. The absorbance was measured at 420 nm. TFC was expressed as Quercetin equivalent (QE) mg/g.

Antioxidant activity

The antioxidant activity, 2-diphenyl-1-picrylhydrazyl (DPPH), of EBP extract was determined according to the method stated by Xu *et al.* (2022). Briefly, 1 mL extract was supplemented with 1 mL of DPPH (0.1 mol/L) solution. The absorbance was determined at 517 nm. The scavenging concentration (IC₅₀) was expressed as mg/mL.

Antibacterial activity of EBP extract: determination of minimum inhibitory concentration

The MIC of EBP extract was assessed against *Staphylococcus aureus* ATCC 6538, *Salmonella enterica* ATCC 14028, *Listeria monocytogenes* ATCC 19117, and *Escherichia coli* ATCC 8739 (Fourati *et al.*, 2019). The final concentrations were 0.187, 0.375, 0.75, 1.5, 3.0, 6.0, and 12.0 mg/mL. Following this, 10 µL of cell suspension containing 10⁶ colony-forming unit (CFU)/mL was introduced into each well. Following the incubation period, the wells were treated with 0.5 mg/mL thiazolyl blue tetrazolium bromide (MTT) solution and incubated at 37°C for 30 min. The mixture in wells remained clear after adding MTT, demonstrating the ceasing of microbial growth.

Analysis of raw minced beef meat

Physiochemical analysis of treated meat

pH analysis: pH of the meat mixture was determined with 1:10 distilled water (w/v) (Smaoui *et al.*, 2016). pH values of filtrates were measured using a pH meter on each sampling day.

Protein and lipid oxidation: Regarding protein oxidation, sulphhydryl (SH), carbonyl content (CC), and metmyoglobin (MetMb) contents were evaluated. Sulphhydryl content in each meat sample was quantified using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) method as described by Wang *et al.* (2018). The absorbance of different mixtures was measured at 412 nm. Sulphhydryl content was evaluated using molar extinction coefficient (13,600 M⁻¹ cm⁻¹) and expressed as mmol SH/g of protein.

Carbonyl content was quantified by following the method described by Mtibaa *et al.* (2019). Carbonyl content was evaluated by molar extinction coefficient (22,000 M⁻¹ cm⁻¹) and was expressed as nmol carbonyl/mg of protein.

MetMb (%) was quantified as described by Wang *et al.* (2018). Each meat sample, 10 g, was homogenized with 50 mL of phosphate buffer K₃PO₄ (pH 6.8, 0.04 M) followed by centrifugation (3000× g) for 30 min. Following filtering, the absorbance of filtrates was assessed at 582,

503, 525, and 557 nm. MetMb (%) was calculated according to the equation described by Wang *et al.* (2018).

Concerning lipid oxidation, peroxide values (PV), conjugated dienes (CD), and thiobarbituric acid reactive substances (TBARS) were evaluated. Peroxide value of all meat samples was quantified by following the Folch method of extraction explained in a study conducted by Mtibaa *et al.* (2019), and the results were expressed as molarity equivalent of peroxide/kg of meat.

Conjugated dienes were quantified by following the protocol described by Mtibaa *et al.* (2019). Conjugated dienes concentration was calculated using molar extinction coefficient ($25,200 \text{ M}^{-1} \text{ cm}^{-1}$), and the results were expressed as $\mu\text{mol/mg}$ of meat.

Thiobarbituric acid reactive substance values were determined by following the method described by Eymard *et al.* (2005). The absorbance of meat samples was evaluated by aspectrophotometer. Results were expressed as mg malonaldehyde (MDA)/kg of meat.

Microbiological analysis

From each sample (control, BHT-treated, and 1-EBP, 2-EBP, and 4-EBP extract-treated), 10 g was mixed with 90 mL of sterilized peptone water in a stomacher, and homogenized. Decimal dilutions were carried out to inoculate the samples into a solid culture medium plate. Aerobic plate counts (APC) and psychrotrophic total counts (PTC) were enumerated using plate count agar (PCA) at 30°C for 48 h (ISO, 2013) and 7°C for 10 days (ISO, 2001), respectively. *Enterobacteriaceae* counts (EC) incubated at 37°C for 24 h in violet red bile lactose agar (ISO, 2004) were performed.

Instrumental color assessment

The colors of the samples were evaluated using a spectrophotometer-colorimetry, MiniScan XETM (Hunter Associates Laboratory Inc., Reston, VA, USA) with a 22-mm aperture and a 10° observer and adjusted with a white tile. Lightness (L^*) component signifies brightness level and ranges from 0 (black) to 100 (white). Redness (a^*) component presents green–red axis, where negative values depict greener shades and positive values indicate redder hues. Yellowness (b^*) component determines blue–yellow axis, where negative values signify bluer tones and positive values indicate yellower shades.

Sensory evaluation

The sensory qualities (odor, color, appearance, and overall acceptability [OA]) of all meat samples were evaluated by 30 non-trained members (15 females, 15 males) of a

panel comprising administrative staff and graduate students of the University of Sfax (Tunisia). The panellists chosen were aged 23–45 years and were non-smokers and regular consumers of beef meat products. Each panellist evaluated three assays of meat samples (control, BHT-treated, and 1-EBP, 2-EBP, and 4-EBP extract-treated) on different days of storage (0, 3, 7, 10, and 14). The assessment employed a hedonic scale ranging from 1 to 9, indicating degrees of dislike (extremely) to like (extremely) (Sucu and Turp, 2018).

Statistical analysis

The samples were analyzed on 0, 3, 7, 10, and 14 days of storage. Further, on each day, three replications were executed. A two-way analysis of variance (ANOVA) was accomplished for all variables. The statistical significance of differences between mean values was analyzed using triplicate measures and Tukey's test was conducted using the Statistical Package for the Social Sciences (SPSS) software.

The PCA method was effectuated to sort out meat samples based on microbial counts, physicochemical analysis, instrumental color, and sensory parameters during refrigerated storage. The PCA type was acquired according to Pearson correlation, the plot type was a correlation biplot, and the coefficients were automatic. This approach was achieved using XLSTAT.

Results and discussion

Phytochemical content of EBP extract

Concentrations of betacyanins and betaxanthins in EBP extracts were 8.10 mg/g and 10.02 mg/g, respectively. Slatnar *et al.* (2015) studied the aqueous red beetroot extract and observed a betalain concentration of 21.93 mg/g. According to Fu *et al.* (2020), changes in betalain concentration were affected by the ratio, time, and temperature of extraction. The EBP extract exhibited a high concentration of TPC (30.21 mg GAE/mL). The findings of this study were higher than those reported by Aykın-Dinçer *et al.* (2021), who discovered a TPC concentration of 27.72 mg GAE/mL in aqueous beetroot pulp extract. The concentration of TFC in EBP extract was 0.413 mg QE/mL. A comparable concentration of TFC (0.490 mg CE/mL) was stated by Sigwela *et al.* (2021). Thus, concentrations of phytochemical compounds in beetroot samples depended on the part, variety, origin, and agricultural conditions of the root (Chaari *et al.*, 2022b).

Biological activities of EBP extract

The EBP extract demonstrated remarkable efficacy in scavenging free radicals, with an IC_{50} value of 0.96 mg/mL. Numerous studies (Chhikara *et al.*, 2019; Guine *et al.*, 2018; Righi Pessoa da Silva *et al.*, 2018) have documented the antioxidant activity of beetroot extract. However, the level of antioxidant activity varied due to multiple factors, such as post-harvest quality, deterioration caused by storage time, physical damage, and elevated temperature. The antioxidant activity of beetroot extract depended not only on the existence of polyphenols but also on the concentration of betalains (Guine *et al.*, 2018).

The MIC of beetroot extract against *L. monocytogenes* and *S. aureus* was 0.75 mg/mL, and it was 1.5 mg/mL against *E. coli* and *S. enterica*. Similar results were demonstrated by Čanadanović-Brunet *et al.* (2011) when ethanolic beetroot extract at 50 mg/mL was tested against *L. monocytogenes* (0.75 mg/mL) and *E. coli* (1.5 mg/mL). In general, Gram-positive bacteria tend to exhibit greater sensitivity to beetroot, compared to Gram-negative bacteria (Kumar and Brooks, 2018; Sadowska-Bartosz and Bartosz, 2021), which causes reduced vulnerability of these bacteria (Breijyeh *et al.*, 2020). Hence, the extract's antibacterial effects are attributed to its rich concentration of phytochemical components, as previous studies have indicated their effective antimicrobial properties (Kchaou *et al.*, 2016; Martinez *et al.*, 2020).

Analysis of raw minced beef meat samples

EBP extract at 0.075%, 0.15%, and 0.3%, equivalent to 1× MIC, 2× MIC, and 4× MIC of *L. monocytogenes* were added to raw minced beef meat.

Microbiological analysis

As presented in Figure 1, the maximum level of APC, recorded by AFNOR (2004), was 6.7 log CFU/g of meat. This value was observed in control samples, followed by BHT treatment. As shown in Figure 1, APC was significantly reduced by adding EBP extract. APC in the meat samples containing 2-EBP and 4-EBP extracts were 6.54 log CFU/g and 6.47 log CFU/g, respectively. Thus, 2-EBP and 4-EBP extracts-treated meat samples maintained APC under the recommended limit (AFNOR, 2004). Additionally, these results revealed a significant decrease in APC with an increase in EBP extract concentration. According to Marrone *et al.* (2021), the 2% and 5% aqueous beetroot extract if applied on meat burgers displayed 7.73 log CFU/g and 7.95 log CFU/g of APC after 6 days. These findings indicated that EBP extract was more efficient in delaying bacterial growth.

An increase in PTC growth in control samples was observed at each sampling time (Figure 2). Remarkably, EBP extracts significantly reduced PTC in meat samples. After 14 days, the PTC of meat samples treated with 1-EBP, 2-EBP, and 4-EBP extracts was 6.56 log CFU/g, 6.42 log CFU/g, and 6.35 log CFU/g, respectively. Interestingly, PTC in meat samples did not attain 6.7 log CFU/g valuation (AFNOR, 2004) when EBP extracts were used. Hence, these extracts allowed an extended shelf life of meat for up to 10 days at 4°C.

All EBP extracts-treated meat samples exhibited lower *Enterobacteriaceae* counts than those of control and BHT-treated samples (Figure 3). *Enterobacteriaceae* count remained within the acceptable limit of 2 log CFU/g for EBP extracts-treated meat samples. On the 14th day, meat samples treated with 1-EBP, 2-EBP,

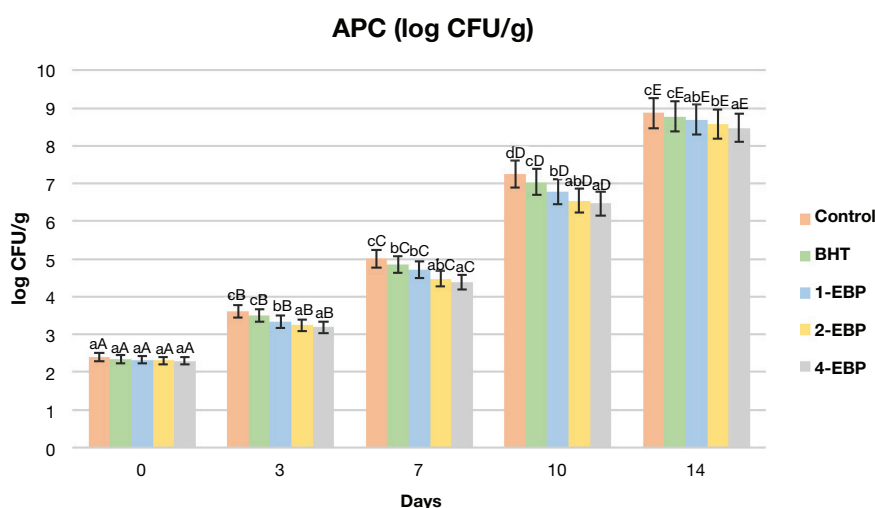


Figure 1. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on APC; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

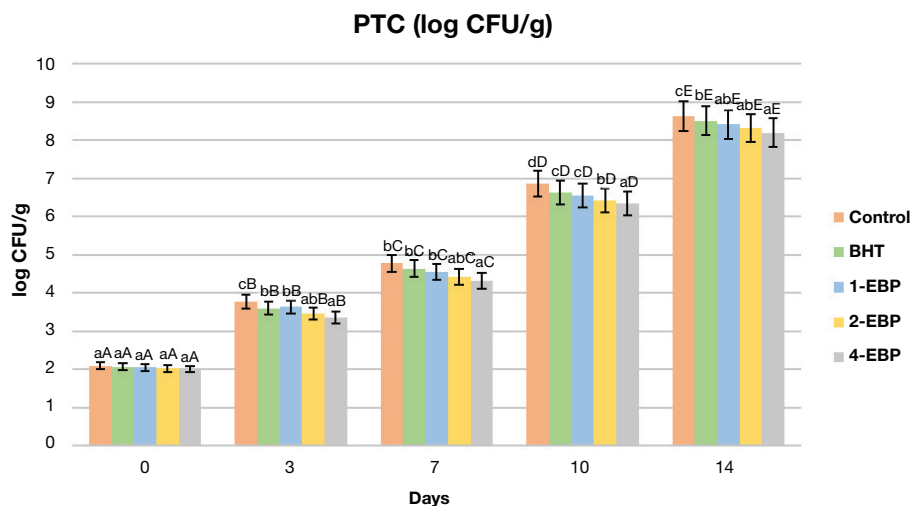


Figure 2. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on PTC; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

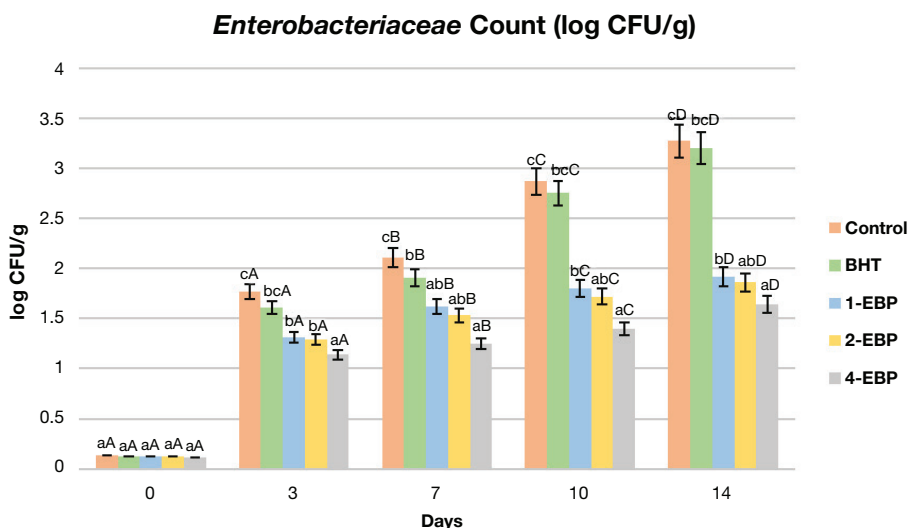


Figure 3. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on *Enterobacteriaceae* count; a–c Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–D Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

and 4-EBP extracts presented 1.92 log CFU/g, 1.86 log CFU/g, and 1.64 log CFU/g of *Enterobacteriaceae* count, respectively (AFNOR, 2004).

Therefore, the influence of EBP extracts was associated with the dosage and duration of storage. Marrone *et al.*'s (2021) results were in agreement with our findings for testing the effect of beetroot powder and aqueous extract on microbial growth in black Angus burgers. The inhibition of bacterial growth in treated meat samples was attributed to betalains and/or phenolic compounds found in beetroot extract (Aykın-Dinçer *et al.*, 2021; Čanadanović-Brunet *et al.*, 2011).

Physiochemical analysis

pH analysis: The results of pH measurement are depicted in Table 1. The pH of the control sample on all days of observation was maximum, followed by the BHT-treated sample. High pH was due to the development of secondary metabolites formed by microorganisms and the degradation of amino acids (Rashidaie Abandansarie *et al.*, 2019). At the same time, the EBP extracts-treated meat samples showed lower pH. Thus, higher concentrations of EBP extract (0.3%) produced better results. Overall, it indicated that the concentration of beetroot extracts significantly impacted the pH of meat samples.

Table 1. pH of the raw minced beef meat treated with BHT (0.01%), 1-EBP (0.075%), 2-EBP (0.15%), and 4-EBP (0.3%) during 14 days of storage.

Samples	Days of storage				
	0	3	7	10	14
Control	5.55 ± 0.244 ^{a,A}	5.75 ± 0.265 ^{b,B}	5.84 ± 0.280 ^{c,B,C}	5.92 ± 0.284 ^{c,C}	6.21 ± 0.304 ^{d,D}
BHT	5.54 ± 0.255 ^{a,A}	5.68 ± 0.261 ^{a,b,B}	5.78 ± 0.277 ^{b,B,C}	5.86 ± 0.281 ^{b,C}	6.13 ± 0.300 ^{c,D}
1-EBP	5.53 ± 0.243 ^{a,A}	5.66 ± 0.260 ^{a,B}	5.76 ± 0.276 ^{a,b,B}	5.84 ± 0.280 ^{b,C}	6.01 ± 0.294 ^{b,D}
2-EBP	5.53 ± 0.238 ^{a,A}	5.64 ± 0.259 ^{a,A,B}	5.73 ± 0.275 ^{a,b,B}	5.81 ± 0.279 ^{b,C}	5.95 ± 0.292 ^{b,D}
4-EBP	5.50 ± 0.242 ^{a,A}	5.61 ± 0.258 ^{a,A,B}	5.68 ± 0.273 ^{a,A,B}	5.71 ± 0.274 ^{a,B}	5.82 ± 0.285 ^{a,C}

±Standard deviation (SD) of three replicates; ^{a-d}mean values that are not followed by a similar letter in the same column differ significantly ($P < 0.05$); ^{A-D}mean values that are not followed by a similar letter in the same line differ significantly ($P < 0.05$).

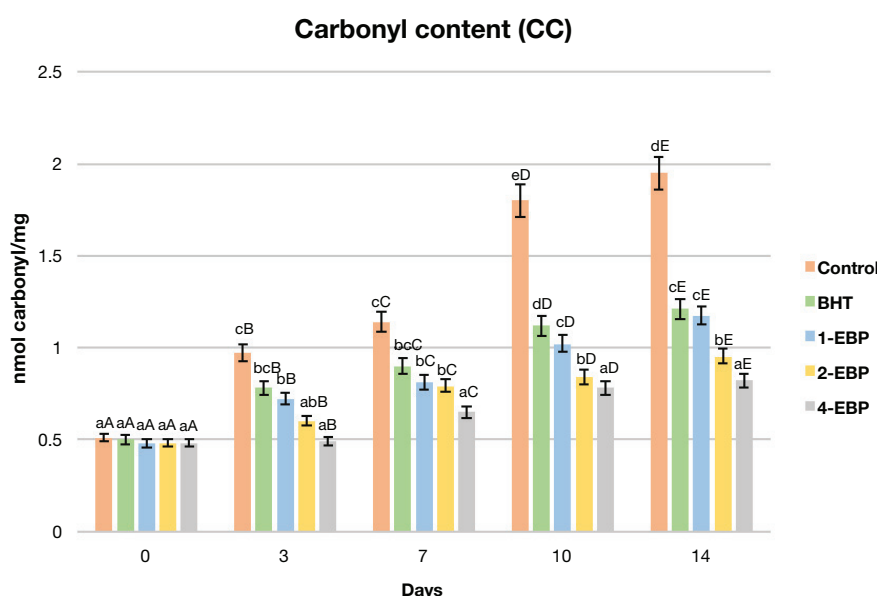


Figure 4. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on carbonyl content (nmol carbonyl/mg of protein) of raw minced beef meat during storage; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

Evaluation of protein oxidation: Throughout the storage period, a noticeable and statistically significant rise in carbonyl content was observed (Figure 4). Control samples presented a higher protein carbonylation level than treated samples. Additionally, EBP extracts were more efficient ($P < 0.05$) than BHT-treated samples in delaying carbonylation. The higher the EBP extract concentration, the lower the protein carbonylation perceived. A similar trend was observed by Bellucci *et al.* (2021) because they incorporated red pitaya extract (0.01%) in pork patties, achieving 5.29 nmol carbonyl/mg of protein after 18 days of storage.

All meat samples showed a significant decrease in sulphhydryl concentration during storage (Figure 5). This decrease could be linked to the formation of disulfide bonds (Lund *et al.*, 2011). The percentage of sulphhydryl linkage breakdown in non-treated meat samples (52.71%)

was higher than in 1-EBP (40.37%), 2-EBP (35.48%), and 4-EBP (27.45%) extracts-treated samples after 14 days of storage. Our results matched the results of Elhadeif *et al.* (2020), who showed a significant difference in sulphhydryl concentration in beef meat treated with *Ephedra* extract.

Values of MetMb increased ($P < 0.05$) significantly, attaining 67.06%, 42.87%, 36.20%, and 30.35% for meat samples treated with BHT, 1-EBP, 2-EBP, and 4-EBP extracts after 14 days (Figure 6). A decrease in a^* was attributed to the conversion of oxymyoglobin to MetMb through oxidation, and 2-EBP and 4-EBP extracts were better at retarding oxymyoglobin oxidation. Protein oxidation was delayed due to the antioxidant property of EBP extract, which prolonged the preservation of the meat's red color. The effectiveness of 4-EBP extract at 0.3% could be attributed to its high content of betalains, which displayed metal chelating activity, and therefore

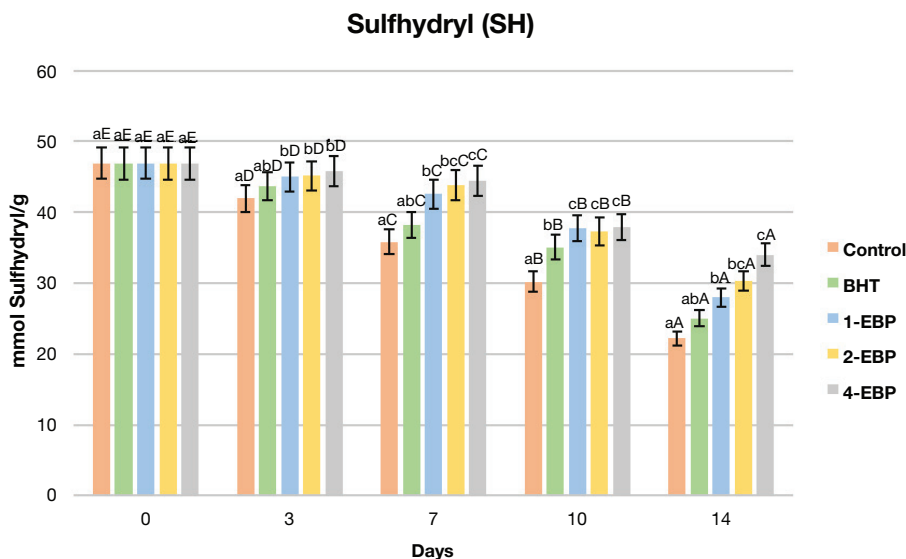


Figure 5. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on sulphhydryl content (mmol sulphhydryl/g) of raw minced beef meat during storage; a–b Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–C Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

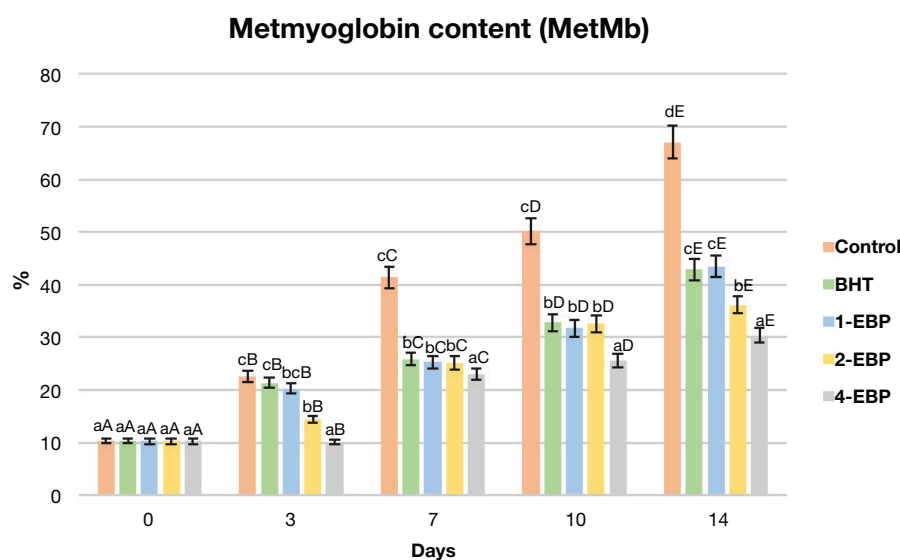


Figure 6. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on MetMb (%) of raw minced beef meat during storage; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

delivered a higher level of protection against protein oxidation (Kumar and Kumar, 2022).

Evaluation of lipid oxidation: Figure 7 shows a substantial ($P < 0.05$) increase in peroxide values across all samples during the storage period. Accordingly, the highest peroxide value was observed in the control sample. Furthermore, on the 14th day, the meat sample treated with 4-EBP extract yielded the lowest peroxide value (6.85 meq O_2 /kg), followed by 2-EBP extract (PV = 7.01 meq O_2 /kg) and 1-EBP extract (PV = 7.35 meq O_2 /kg).

These findings matched well with the results reported by Fourati *et al.* (2020), who treated beef meat with 1% ethanolic pomegranate peel extract. The average peroxide value of all meat samples was less than 25 meq O_2 /kg, the generally acknowledged upper threshold for fatty food products (Sallam *et al.*, 2004).

In all samples, a significant increase ($P < 0.05$) in conjugated dienes was observed for 3 days (Figure 8). This was explained by the fact that conjugated dienes were formed faster than the decomposing of hydroperoxides.

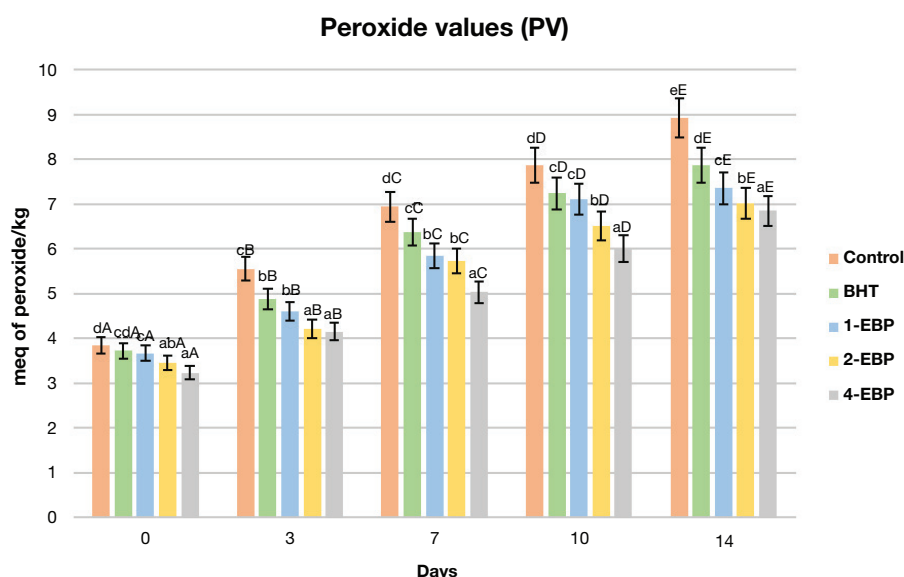


Figure 7. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on peroxide value (meq of peroxide/kg) of raw minced beef meat during storage; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

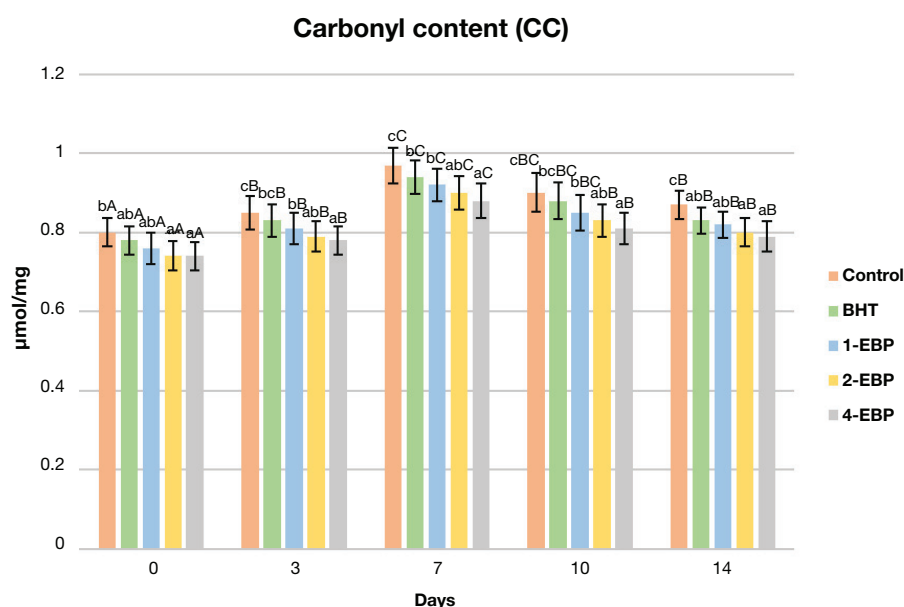


Figure 8. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on conjugated dienes (µmol/mg) of raw minced beef meat during storage; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

A decrease in conjugated dienes could be elucidated by the formation of secondary lipid oxidation products, specifically aldehydes and ketones (Domínguez *et al.*, 2019). Moreover, a decrease in conjugated dienes of meat samples treated with 1-EBP, 2-EBP, and 4-EBP extracts was observed during storage. This proved that EBP extracts might have a higher potential to reduce lipid oxidation by delaying conjugated dienes formation in meat products.

As shown in Figure 9, TBARS values increased significantly ($P < 0.05$) in all treated meat samples during storage. On day 0, comparable TBARS values ($P > 0.05$) were observed in all meat samples. By the 14th day of storage, higher TBARS values were observed for the control (2.86 mg MDA eq/kg of meat) sample and those treated with BHT (2.36 mg MDA eq/kg of meat). These values exceeded the specified limit of TBARS (2 mg MDA eq/Kg of meat; Elhadeef *et al.*, 2021). TBARS levels of 1-EBP,

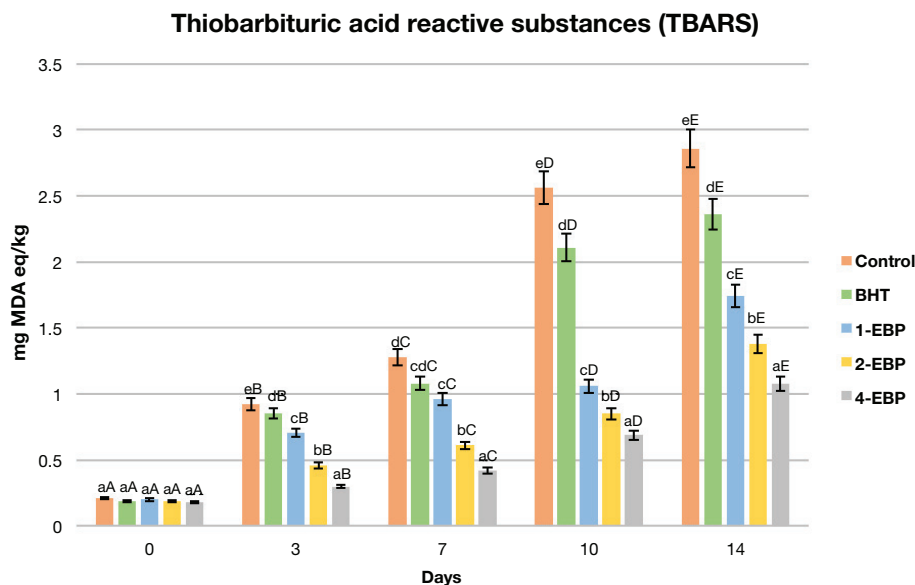


Figure 9. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on TBARS (mg MDA-eq/kg) of raw minced beef meat during storage; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

2-EBP, and 4-EBP extracts-treated meat samples were 1.74, 1.38, and 1.08 mg MDA eq/kg, respectively. TBARS levels exhibited a notable decrease with an increase in EBP extract concentration. Bellucci *et al.* (2021) established that 0.1% of red pitaya extract decreased TBARS value in pork parties to 1.25 mg MDA eq/kg of meat after 18 days of storage. Given that betacyanin is the primary pigment responsible for the antioxidant activity found in red pitaya (Roriz *et al.*, 2022), it is worth mentioning that several studies have mentioned the potential utilization of natural extracts as alternatives to synthetic additives. These natural extracts have demonstrated the ability to delay lipid oxidation processes effectively, comparable to or surpassing the performance of synthetic additives (Echegaray *et al.*, 2023; Smaoui *et al.*, 2021).

Meat color analysis

According to Table 2, L^* , a^* , and b^* decreased ($P < 0.05$) significantly with the progression of storage time. Color indexes of EBP extracts-treated meat samples (0.075%, 0.15%, and 0.3%) were significantly higher ($P < 0.05$) than those of control and BHT-treated meat samples. The decrease in color indexes could be due to lipid oxidation. Hydroperoxides and other reactive oxygen species, generated as primary lipid oxidation products, are responsible for oxidizing ferrous iron (Fe^{2+}) from OxyMb to ferric iron (Fe^{3+}) in MetMb, as established in a previous study (Estévez *et al.*, 2020). L^* of all meat samples decreases during storage. A similar trend was observed by Marrone *et al.* (2021) when they added beetroot extract B1 (0.8%) and B2 (2%) in beef meat.

Concerning a^* values, the inclusion of EBP extract significantly ($P < 0.05$) increased the redness of meat samples, compared to other samples, as elucidated by the extract's color. Furthermore, 1-EBP, 2-EBP, and 4-EBP extracts enhanced meat's red color even after 14 days of storage with respective a^* values of 12.02, 13.74, and 13.86. Comparable trends were stated by Marrone *et al.* (2021) when they treated beef meat burgers with 2% and 5% beetroot extracts. These outcomes demonstrate that the existing betalain content is highly effective in providing meat with the proper red color. Hence, an elevated concentration of EBP extract can lead to an augmentation of redness.

Beetroot extract preserved the color of beef meat (Marrone *et al.*, 2021) and Turkish fermented beef sausage (Sucu and Turp, 2018). Regarding b^* values of non-treated meat samples, a decline was observed, reaching the lowest value of 13.17 on the 14th day of storage, compared to the treated meat samples, which showed a slight decrease in b^* . Otherwise, b^* values of meat samples treated with 0.075%, 0.15%, and 0.3% EBP extracts were higher than those treated with 0.01% BHT. This fact was evident in the study conducted by Palmeri *et al.* (2018), which reported that the same trend in a^* and b^* values was observed when the authors added prickly pear extract (PPE) to sliced beef meat.

Sensory assessment

The development of desirable odor and color determines the sensory quality of meat, and the inclusion of EBP

Table 2. Color parameters of raw minced beef meat treated with BHT and 1-EBP, 2-EBP, and 4-EBP extracts during 14 days of storage.

		Days of storage				
Samples		0	3	7	10	14
L*	Control	45.12 ± 1.985 ^{a,E}	45.19 ± 2.034 ^{b,D}	45.08 ± 2.074 ^{a,C}	44.78 ± 0.149 ^{a,B}	43.43 ± 2.172 ^{a,A}
	BHT	45.15 ± 1.986 ^{a,E}	44.82 ± 2.017 ^{c,D}	44.60 ± 2.052 ^{b,C}	44.24 ± 2.124 ^{b,B}	43.17 ± 2.159 ^{b,c,A}
	1-EBP	45.20 ± 1.988 ^{b,E}	44.61 ± 2.007 ^{b,c,D}	44.33 ± 2.039 ^{b,c,C}	43.64 ± 2.095 ^{c,B}	42.36 ± 2.118 ^{c,A}
	2-EBP	45.18 ± 1.987 ^{a,b,E}	44.20 ± 1.989 ^{b,D}	43.77 ± 2.013 ^{d,C}	42.92 ± 2.060 ^{d,B}	41.15 ± 2.058 ^{d,A}
	4-EBP	45.21 ± 1.989 ^{b,E}	44.11 ± 1.985 ^{a,D}	42.52 ± 1.995 ^{c,d,C}	41.17 ± 1.976 ^{a,B}	40.03 ± 2.002 ^{b,A}
a*	Control	12.36 ± 0.544 ^{a,C}	12.13 ± 0.546 ^{a,C}	11.05 ± 0.508 ^{a,B}	11.32 ± 0.543 ^{a,B,C}	10.14 ± 0.507 ^{a,A}
	BHT	12.40 ± 0.546 ^{a,C}	12.21 ± 0.549 ^{a,C}	11.96 ± 0.550 ^{a,B}	11.41 ± 0.548 ^{a,A,B}	11.07 ± 0.554 ^{b,A}
	1-EBP	14.47 ± 0.637 ^{b,D}	13.78 ± 0.620 ^{b,C}	13.04 ± 0.600 ^{b,B}	12.17 ± 0.584 ^{b,A}	12.02 ± 0.601 ^{c,A}
	2-EBP	16.22 ± 0.714 ^{c,D}	15.96 ± 0.718 ^{c,C}	14.83 ± 0.682 ^{c,B}	14.07 ± 0.675 ^{c,A,B}	13.74 ± 0.687 ^{d,e,A}
	4-EBP	17.56 ± 0.773 ^{d,E}	16.68 ± 0.751 ^{d,D}	15.83 ± 0.728 ^{d,C}	14.97 ± 0.719 ^{c,B}	13.86 ± 0.693 ^{b,A}
b*	Control	19.45 ± 0.856 ^{c,E}	18.72 ± 0.842 ^{c,D}	17.48 ± 0.804 ^{a,C}	15.04 ± 0.722 ^{a,B}	13.17 ± 0.659 ^{a,A}
	BHT	18.89 ± 0.831 ^{a,E}	18.50 ± 0.833 ^{b,D}	17.76 ± 0.817 ^{b,C}	16.41 ± 0.788 ^{b,B}	14.78 ± 0.739 ^{b,A}
	1-EBP	19.32 ± 0.850 ^{b,D}	18.15 ± 0.817 ^{a,C}	17.82 ± 0.820 ^{b,c,B,C}	17.19 ± 0.825 ^{c,B}	15.48 ± 0.774 ^{c,A}
	2-EBP	18.78 ± 0.826 ^{a,E}	18.45 ± 0.830 ^{a,b,D}	17.91 ± 0.824 ^{c,C}	17.31 ± 0.831 ^{c,d,B}	16.07 ± 0.804 ^{d,A}
	4-EBP	19.43 ± 0.855 ^{b,c,D}	18.54 ± 0.834 ^{b,C}	18.06 ± 0.831 ^{d,C}	17.47 ± 0.839 ^{d,B}	16.76 ± 0.838 ^{e,A}

Standard deviation (SD) of three replicates; ^{a–d}mean values that are not followed by a similar letter in the same column differ significantly ($P < 0.05$); ^{A–E}mean values that are not followed by a similar letter in the same line differ significantly ($P < 0.05$).

extract boosted its overall acceptability. Color attributes are crucial quality indicators for consumers to accept or reject a particular food (Martins *et al.*, 2017). The appearance, color, odor, and overall acceptability scores of beef meat incorporating EBP extracts were better than those of control and BHT-treated samples (Figures 10A–C). These observations are because the addition of EBP extract imparted a naturally vibrant pink hue to the treated meat samples (Figure 10B). Our results are in line with the results of Aykın-Dinçer *et al.* (2021). These authors indicated that sausages treated with 0.052% and 0.1% beetroot extract had a better consumer acceptance score than control sausages. Furthermore, 1.0% or 1.2% lyophilized red beet water extracts (LRBWE) were effective on the sensory quality of Turkish pastirma during storage at 4°C for 5 months (Aksu *et al.*, 2020). As shown in Figure 10, all sensory attributes diminished significantly ($P < 0.05$) during refrigerated storage. This reduction could be due to enhanced oxidation and microbial growth during storage. Based on these results, it can be inferred that incorporating EBP extract as a colorant in meat samples could improve their sensory characteristics.

Principal component analysis

The PCA method was employed to acquire a general overview of differences and similarities between five samples at four periods. Score plots showing the dispersion of various samples that follow principal components were generated using PCA. Figures 11A,B reveal that the major principal component (F1) contributed to 85.88% of

total variances, while F2 contributed to 6.23% of variance in the data. According to the loading plot, both the first (F1) and second (F2) principal components collectively provided the highest data variation (92.11%). Moreover, the score plot (Figure 11A) displayed a noticeable distinction in 25 samples.

Lipid and protein oxidation parameters, excluding sulphhydryl, microbial counts, and pH, positively influenced F1. At the same time, these factors were inversely correlated with sensory assessment and instrumental color measurements (Figure 11B). This observation aligns with the findings of Wang *et al.* (2021), who established a clear causal relationship between myoglobin autoxidation and the stability of meat color. The primary cause of deterioration in meat color is an oxidative reaction, primarily associated with structural and chemical alterations in myoglobin. Additionally, the correlation observed between sulphhydryl levels and color measurements concurs with the research conducted by Estévez *et al.* (2020), emphasizing the protective role of sulphhydryl against oxidation and its impact on the quality of meat color. Furthermore, several studies, including Maqsood *et al.* (2015), have pointed out that lipid oxidation in meat leads to adverse changes in sensory properties, resulting in reduced consumer acceptability and the emission of undesirable off-odors. This phenomenon could be attributed to the heightened susceptibility of phospholipid fraction to oxidation, as indicated by Fourati *et al.* (2020). Microbial metabolism favors the breakdown of proteins, releasing amino acids and converting aldehydes to malonaldehyde (Pellissery *et al.*, 2020). This enzymatic

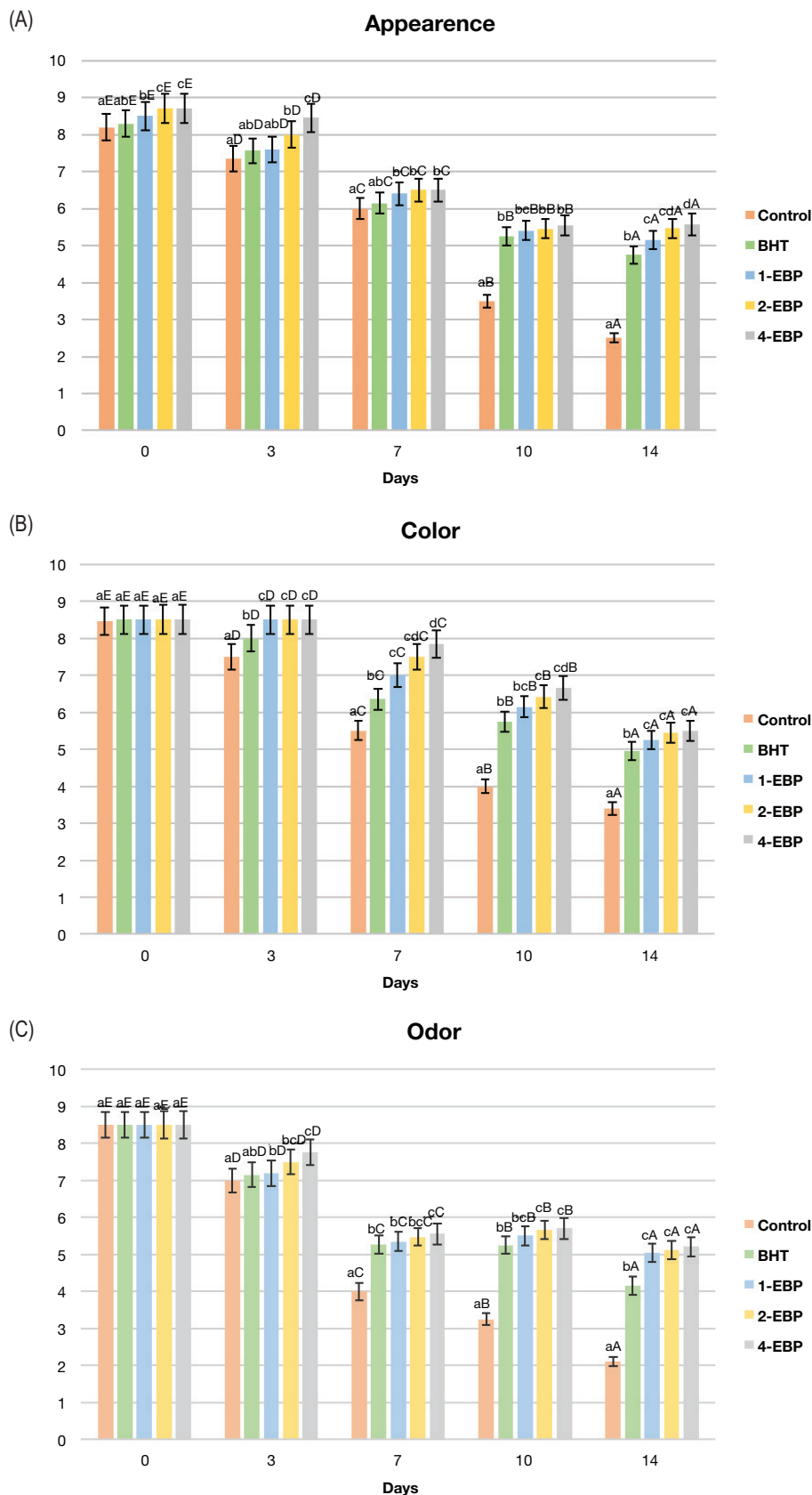


Figure 10. Effect of BHT and 1-EBP, 2-EBP, and 4-EBP extracts on the (A) appearance, (B) color, (C) odor, and (D) overall acceptability (OA) of raw minced beef meat during storage; a–d Mean values for each treatment with a different letter are significantly different ($P < 0.05$). A–E Mean values for each storage day with a different letter are significantly different ($P < 0.05$).

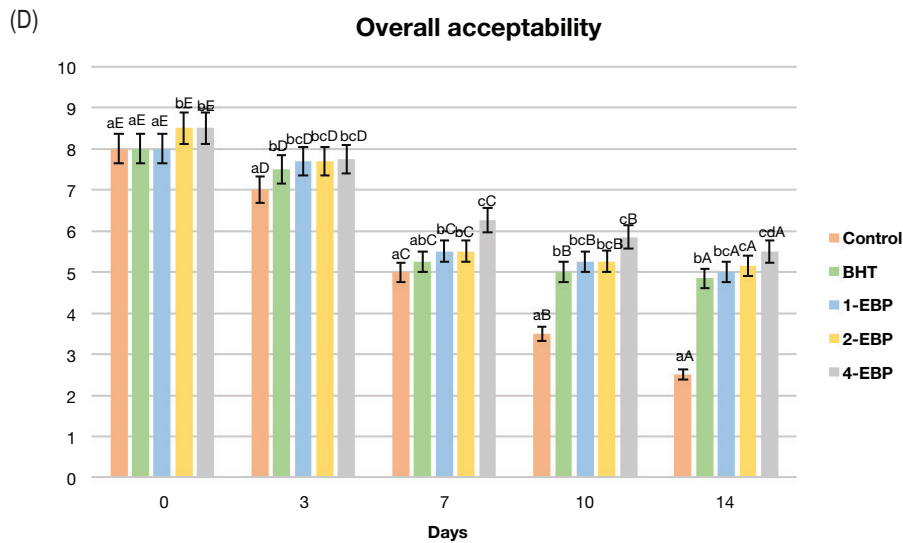


Figure 10. (Continued)

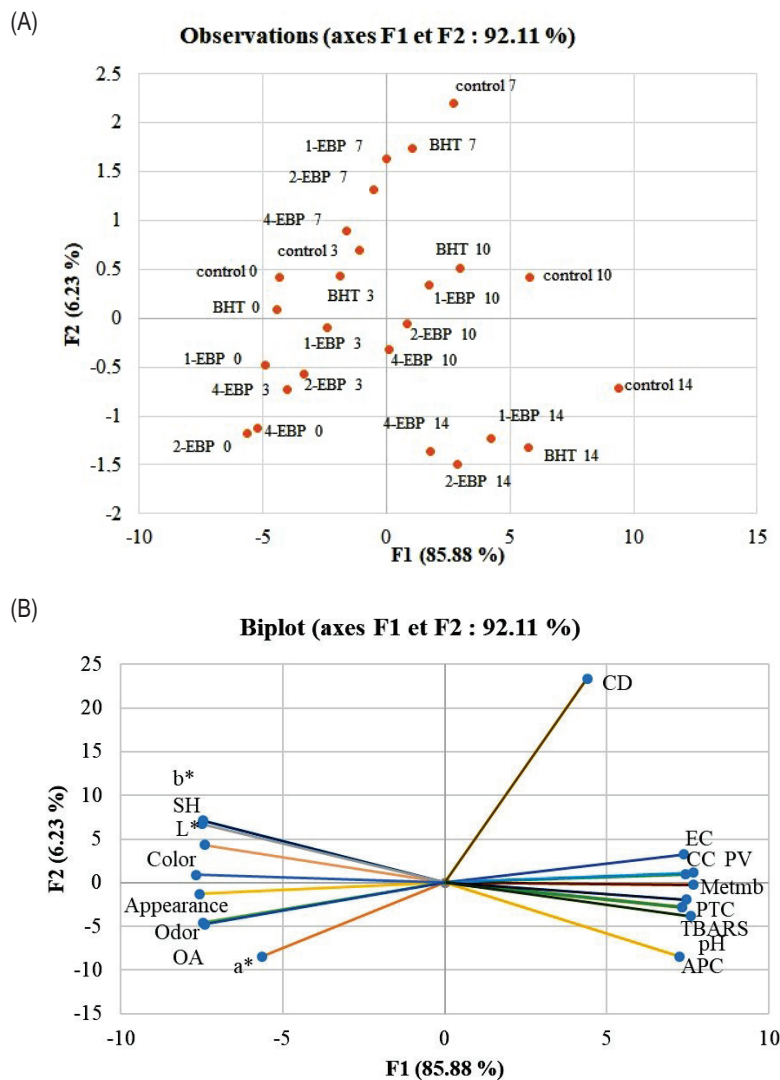


Figure 11. Score plots of two principal components (F1 and F2) based on (A) all samples and (B) microbial counts, physico-chemical properties, instrumental colors, and sensorial attributes of meat samples.

breakdown of amino acids increases pH levels (Carvalho *et al.*, 2017; Guo *et al.*, 2020). Mojaddar Langroodi *et al.* (2021) depicted the connection between sensory and microbiological attributes, linking elevated microorganism levels and lipid oxidation to unsatisfactory sensory characteristics, including off-odors, off-colors, and an undesirable visual appearance. Interestingly, chemometric techniques, such as PCA, are widely utilized to evaluate the quality of meat, referring to its oxidative, microbiological, and color stability during storage (de Farias Marques *et al.*, 2022; Elhadeif *et al.*, 2023).

Conclusion

Findings revealed that EBP extracts displayed antioxidant, antibacterial, and colorant properties. The extracts contained high levels of phenolic and flavonoid contents. In addition, these displayed a notable positive impact on meat quality. 4-EBP extract with a concentration of 0.3% was the best treatment, with maximum impact on delaying chemical oxidation and microbial growth. Therefore, the shelf life was extended by approximately 14 days under 4°C. Additionally, color parameters, including sensory properties, were positively affected by a high concentration of these pigments. Concerning PCA, this analysis proved a correlation between sensory attributes and instrumental color measurements, lipid/protein oxidation, and microbial counts. Consequently, EBP extract could be used as a sustainable natural colorant and exploited as an alternative to synthetic colorants in the meat industry.

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