



Effect of grape pomace on oxidative and microbial quality of chill-stored beef model systems

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ABSTRACT

Grape pomace, a by-product of winemaking, is rich in bioactive compounds and has recently attracted attention for its potential application in functional foods. In this study, grape pomace powder (GPP) derived from the local Kösetevék grape variety was incorporated into beef model systems at concentrations of 0.5 %, 0.75 %, and 1.0 % to evaluate its effects on oxidative and microbial stability. GPP supplementation significantly reduced lipid oxidation and metmyoglobin formation, and it also decreased lightness and yellowness values, resulting in a darker appearance in meat batters ($p < 0.001$). However, the 1.0 % GPP group did not provide additional oxidative protection compared to the 0.5 % and 0.75 % groups. Textural attributes such as hardness and chewiness were affected by GPP, with the 1.0 % group exhibiting the highest chewiness values ($p < 0.001$). Although microbial counts were lower in GPP-treated samples than in the control, all samples exceeded acceptable spoilage thresholds by day 6. Overall, GPP at concentrations up to 1.0 % may serve as a natural additive to improve the shelf life and functional quality of meat products.

1. Introduction

Meat and meat products are important in human nutrition due to their essential fatty acids and highly bioavailable proteins. However, spoilage processes like microbial growth, lipid oxidation, and protein oxidation negatively affect shelf life and consumer acceptance. Lipid oxidation is a fundamental chemical reaction in meat that leads to undesirable changes in flavor, odor, color, texture, and nutritional value, and it is also associated with the formation of toxic compounds (Dragoev, 2024). This reaction significantly diminishes the physico-chemical and sensory quality of meat, thereby shortening its shelf life. Therefore, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are frequently used in fresh meat products to control oxidation (Hadidi et al., 2022). These compounds are primarily utilized in meat and meat products to prevent oxidative spoilage, extend shelf life, and maintain color stability (Alexandre et al., 2022).

However, in recent years, serious concerns have been raised regarding the safety of synthetic antioxidants. Studies have reported that high doses of exposure to these compounds may lead to genotoxic, carcinogenic, and toxic effects (Ji et al., 2024). Although BHT and BHA are effective in preventing lipid oxidation, they have been shown to possess potential endocrine-disrupting effects, and long-term

consumption has been associated with certain types of cancer (Ji et al., 2024; Sun et al., 2024). These findings have significantly increased interest in natural antioxidants within the food industry. Accordingly, there has been a growing number of studies in recent years investigating the use of spices, aromatic herbs, fruit peels and pomace, and their extracts as natural preservatives in foods due to their antioxidant properties (Andrés et al., 2017; Karşlıoğlu et al., 2024; Muñoz-Bas et al., 2024).

Winemaking is a significant agri-industrial activity both globally and in Türkiye, and this process generates a considerable amount of by-products. The primary by-product of winemaking is grape pomace, also known as wine pomace. This solid residue is obtained after the pressing of grapes and consists mainly of seeds, skins, stem fragments, and pulp residues. These by-products are generally considered waste and are commonly used for wine alcohol production, animal feed, or fertilizer (Antonić et al., 2020). However, grape pomace is rich in biologically active compounds such as polyphenols, flavonoids, antioxidants, and dietary fibers (Milinčić et al., 2021; Silva et al., 2020).

Owing to these properties, grape pomace presents a safer and more sustainable alternative to synthetic antioxidants and holds significant potential for the development of functional foods and nutraceutical products (Lopes et al., 2025). Indeed, recent studies have shown that grape pomace can be processed through extraction and drying to yield

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natural compounds with high antioxidant content. The antioxidant and antimicrobial effects of polyphenols present in these by-products have been extensively investigated (Andrés et al., 2017; Cilli et al., 2020; de Alencar et al., 2022; Garrido et al., 2011; Ryu et al., 2014).

These effects are attributed to the ability of phenolic compounds to scavenge free radicals, donate hydrogen atoms, and chelate metal ions (Milinčić et al., 2021). Additionally, these compounds have been reported to exhibit antimicrobial activity by inducing morphological changes in microorganisms and disrupting the integrity and permeability of bacterial cell walls (Andrés et al., 2017). However, it has also been noted that interactions between these bioactive compounds and proteins, lipids, and minerals present in meat may reduce their antioxidant and antimicrobial efficacy (Awad et al., 2022). Therefore, optimizing the incorporation level of grape pomace in meat products is essential to ensure the effectiveness and sustainability of its functional properties. This study investigates the potential use of Kösetevik grape pomace—a local red wine grape variety native to Türkiye—as a natural preservative in a model meat system will be investigated due to its antioxidant and antimicrobial properties. In addition, the preservative efficacy of grape pomace will be comparatively evaluated against butylated hydroxytoluene (BHT), a commonly used synthetic antioxidant. Notably, studies examining the application of by-products derived from local Turkish wine grape varieties such as Kösetevik in model meat systems remain quite limited in the literature.

2. Materials and methods

2.1. Experimental location and chemicals

This study was conducted at the Meat Science and Technology Laboratory, Department of Food Engineering, Ankara University. All chemicals and reagents used were of analytical grade and purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Steinheim, Germany). Chemicals used for the phenolic profile analysis were of chromatographic grade, while the others were of analytical grade.

2.2. Preparation and characterization of grape pomace powders

Grape pomace obtained from the local Kösetevik grape (*Vitis vinifera* L.), remaining after fermentation and pressing during red wine production, was sourced from Kavaklıdere A.Ş. This pomace consisted of grape skins, seeds, and stems. The residues were transferred into sterile containers and stored at -18 ± 2 °C until further processing. The pomace was freeze-dried under a vacuum of 0.160 mBar for 72 h using a freeze dryer (FreeZone 2.5, Labconco, Kansas City, MO, USA). The dried material was ground using a spice grinder (Nima Electric Grinder, China) and passed through a 300 µm mesh sieve to ensure particle size uniformity. The final product, referred to as grape pomace powder (GPP), was stored under light-protected conditions until use in model meat systems. All analyses, including chemical composition, color measurements, and microbiological tests were performed directly on grape pomace powder (GPP). Ethanolic extracts of GPP were prepared only for the determination of total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH), and LC MS/MS based phenolic profiling. The components of the GPP samples, including moisture (AOAC 925.10), fat (AOAC 920.85; determined by Soxhlet extraction), protein (AOAC 920.87; determined by the Kjeldahl method), ash (AOAC 923.03), crude fiber (AOAC 978.10), and titratable acidity (AOAC 942.15), were determined according to AOAC (2000) procedures. Water activity (aw) was measured at 25 °C using a LabMaster-aw instrument (Novasina AG, Switzerland), following AOAC Method 978.18. Carbohydrate content (%) was calculated by difference, using the following formula: Carbohydrate (%) = 100 - (Moisture% + Ash% + Fat% + Protein%).

2.3. Preparation of GPP extract

The GPP extract was obtained using the method described by de Alencar et al. (2022). Twenty grams of GPP were soaked in 100 mL of 80 % ethanol solution (80:20, v/v; ethanol and Milli-Q water, LC-MS grade) under light-protected conditions. The mixture was shaken at 190 rpm for 120 min at 25 ± 3 °C (Nüve, NF 400, Ankara, Turkey). After extraction, the supernatant was homogenized using an ultrasonic bath (40 kHz, 60 min) (JP Selecta S.A., SN: 569331, Barcelona, Spain) at room temperature in the dark to ensure uniform distribution of phenolic compounds remaining in the liquid phase. A portion of the obtained supernatant was reserved for TPC, TFC, and DPPH analyses and stored at -18 °C until analysis. A portion of the obtained supernatant was separated for TPC, TFC, and DPPH analyses and stored at -18 °C until analysis. For the analysis of phenolic acid composition, the remaining extract was filtered through Whatman No. 41 filter paper, evaporated at 50 °C \pm 2 °C using a rotary evaporator (Heidolph Laborata 4003, Germany), and subsequently dried at 50 °C \pm 5 °C for 12 h. The dried extracts were stored at -18 ± 2 °C in the dark until further analysis.

2.4. Total phenolic, flavonoids content and DPPH in GPP

The total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method as described by Pu et al. (2018), with minor modifications. The GPP extract was diluted 1:100 with distilled water. An aliquot of 100 µL of the diluted extract was transferred into a test tube, followed by the addition of 500 µL of Folin-Ciocalteu reagent. After 5 min of incubation at room temperature, 500 µL of sodium carbonate solution (Na_2CO_3 , 0.943 mol/L) was added. The samples were then kept in the dark for 2 h, and absorbance was measured at 746 nm using a UV-visible spectrophotometer. Results were expressed as milligrams of gallic acid equivalents (mg GAE/100 g dry GPP).

The total flavonoid content (TFC) was measured using a modified colorimetric method described by Pu et al. (2018). A 500 µL aliquot of the diluted extract was mixed with 50 µL of sodium nitrite solution (0.725 mol/L) and allowed to stand for 6 min at room temperature. Then, 50 µL of aluminium nitrate solution (0.469 mol/L) was added, followed by another 6-min incubation. Finally, 400 µL of sodium hydroxide solution (1 mol/L) was added. After standing for 20 min at room temperature, the absorbance was measured at 502 nm using a UV-visible spectrophotometer. Results were expressed as milligrams of catechin equivalents (mg CTE/100 g dry GPP).

Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, based on a modified method by Ruberto et al. (2007). A freshly prepared ethanolic DPPH solution (0.1 mM) was used as the radical source. The GPP extract, previously diluted 1:4 with distilled water, was added to test tubes in volumes of 10, 20, 30, 40, and 50 µL. Each tube received 2 mL of DPPH solution. The mixtures were shaken for 15 min and incubated in the dark at 25 °C for 5 h. Absorbance was measured at 515 nm using a UV-visible spectrophotometer. The percentage of DPPH inhibition was calculated for each concentration, and the EC_{50} value (µg/g dry GPP) was determined as the concentration of extract required to inhibit 50 % of the DPPH radicals.

2.5. Phenolic profile of GPP by LC-MS/MS

Phenolic compounds were identified and quantified using liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS, Thermo Scientific/TSQ Quantum Access Max, Milford, MA, USA). Prior to injection, the dried extracts (0.25 g) were dissolved in 10 mL of a 0.85 % phosphoric acid-methanol mixture (50:50, v/v), resulting in a final concentration of 25 mg/mL. After filtration through a 0.45 µm cellulose acetate syringe filter, the solutions were stored at 4 °C until analysis. The mobile phase consisted of 0.1 % formic acid (Solvent A) and methanol (Solvent B), with a flow rate set at 0.7 mL/min. The instrumental

settings and chromatographic conditions used in the LC-MS/MS analysis are presented in Supplementary Data [Table S1](#). Phenolic compounds were identified based on their retention times and MS/MS fragmentation patterns compared with authentic standards. For each compound, the parent ions (m/z), product ions, collision energies (CE), and ionization modes (ESI \pm) are listed in Supplementary Data [Table S2](#). These MS/MS transitions reflect the characteristic fragmentation profiles used to confirm compound identities. Quantification was carried out using external calibration curves generated from standard phenolic compounds at different concentrations. Calibration data, including calibration equations, correlation coefficients (R^2), linear ranges, limits of detection (LOD), limits of quantification (LOQ), and relative standard deviations (RSD), are provided in Supplementary Data [Table S3](#). This study targeted the following phenolic compounds; among them 12 were identified and quantified: gallic acid, protocatechuic acid, protocatechuic aldehyde, sesamol, gentisic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, vanillin, syringic acid, p-coumaric acid, taxifolin, ferulic acid, salicylic acid, 4-hydroxybenzoic acid, hesperidin, rosmarinic acid, oleuropein, luteolin-7-glucoside, rutin, resveratrol, ellagic acid, cinnamic acid, naringenin, quercetin, luteolin, apigenin, pinocembrin, chrysin, galangin, and flavone. Results are expressed as $\mu\text{g/g}$ dry GPP.

2.6. Preparation of beef model systems

In the preparation of the model system, matured beef obtained from cattle slaughtered by Beşyıldız Company, located in Sivas, Türkiye, was used as the raw material. Beef model systems were prepared following the method described by [He and Shahidi \(1997\)](#). The formulation consisted of ground beef, deionized water, and either synthetic butylated hydroxytoluene (BHT) or GPP as the antioxidant source. In all formulations, ground beef was mixed with deionized water at 20 % (w/w). Prior to incorporation into the meat mixture, GPP was dispersed in deionized water using a Waring Blender (Model 8011G, Waring Commercial, Torrington, CT, USA) at approximately 18,000 rpm for 1 min to ensure homogeneity. The resulting mixture was then combined with ground beef. BHT was added directly to the meat batter in its commercial powdered form at a concentration of 0.01 g per 100 g of fat, based on the fat content of the meat, in accordance with the maximum allowable usage level specified by the Turkish Food Regulation ([Turkey, 2023](#)).

The beef model systems were formulated with 80 % minced meat and 20 % deionized water. No antioxidant was added to the control group, while the BHT group was supplemented with 0.0025 % BHT, corresponding to 0.01 g per 100 g of fat, based on the fat content of the meat. In the GPP groups, grape pomace powder was used as a natural antioxidant source at levels of 0.5 %, 0.75 %, and 1.0 %, respectively. These inclusion levels were determined based on preliminary trial results and relevant findings from the literature. All formulations were standardized to 100 %, and based on this composition, five different meat batter groups were prepared. To maintain formulation balance, the amounts of minced meat were proportionally reduced to accommodate the added antioxidant ingredients (BHT and GPP).

All meat systems were homogenized for 3 min at approximately 18,000 rpm using a Waring blender, with intermittent manual stirring using a glass rod. Meat batters from each group were then formed into 25-g portions with dimensions of $4 \times 4 \times 1.0$ cm, resulting in a total of 40 meat batter samples. The prepared samples were placed in polystyrene (PS) foam trays, wrapped with PVC film under aerobic conditions, and stored at 4 °C for 6 days under refrigerated conditions. All batches were prepared in two independent replications at different times, and duplicate samples were analyzed from each replication. Samples were collected on days 0, 2, 4, and 6 of storage for physical, chemical, and microbiological analyses, and all analyses were performed immediately after sampling. The initial chemical composition of the beef used in these formulations (wet basis) was as follows: 58.54 %

moisture, 16.21 % protein, and 25.00 % fat.

2.7. Moisture content, pH, and weight loss

The moisture content of the meat batters was analyzed throughout the storage period in accordance to AOAC standard method 950.46 ([AOAC, 2000](#)). Samples were dried at 105 °C in an oven (Nüve FN055, Ankara, Turkey) until a constant weight was reached. The pH of the batters was determined using a digital pH meter (Hanna HI 221, Ann Arbor, USA) after homogenizing 10 g of sample in 100 mL of distilled water. Weight loss (%) was calculated using Equation (1), by comparing the sample weights before and after storage, following [AOAC \(2000\)](#) procedures.

$$\text{Weight loss (\%)} = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}} \quad (1)$$

W_{initial} : Weight of the sample before storage (g)

W_{final} : Weight of the sample after storage (g)

Weight Loss (%): Percentage of weight loss.

2.8. Colorimetric analysis

The surface color of both GPP and meat batter samples was measured using a Minolta CR-500 colorimeter (Minolta Co., Ltd., Osaka, Japan), calibrated with a white standard plate (reference number: 1353123; $Y = 92.70$, $x = 0.3133$, $y = 0.3193$). Color measurements were recorded as L^* (lightness), a^* (redness), and b^* (yellowness) values according to the CIELAB system. For each sample, readings were taken at four points: two on the upper surface and two on the lower surface.

2.9. Lipid oxidation analysis

TBARS values of meat batters were determined during refrigerated storage following the method of [Mielnik et al. \(2006\)](#), with modifications to reduce GPP-related spectral interferences based on [Ganhão et al. \(2011\)](#). Briefly, 15 g of sample was homogenized in 45 mL of 7.5 % TCA, centrifuged (10,000 rpm, 5 min), and filtered. The filtrate was distilled, and 50 mL of distillate was collected. Aliquots (5 mL) were mixed with 5 mL of 0.02 mol/L TBA, incubated at 100 °C for 35 min, cooled, and absorbance was measured at 532 nm. Results were expressed as μg malondialdehyde (MDA) per kg of sample, using 1,1,3,3-tetraethoxypropane (TEP) as the standard.

2.10. Measurement of metmyoglobin

Metmyoglobin (MetMb) content in meat batter samples was measured throughout the storage period using the method described by [Kannan et al. \(2001\)](#). Approximately 5 g of sample was homogenized with 25 mL of cold phosphate buffer (pH 6.8, 40 mM, 4 °C) using an Ultra-Turrax homogenizer (IKA T25, Germany) at 13,500 rpm for 10 s. The homogenates were then stored at 4 °C for 1 h, followed by centrifugation at 5000 rpm for 30 min at 4 °C. The resulting supernatants were filtered through Whatman No. 1 filter paper, and the absorbance of the filtrates was measured at 700, 572, and 525 nm using a UV-VIS double-beam spectrophotometer (Lambda 35, PerkinElmer, USA). The results were expressed as the percentage (%) of metmyoglobin (MetMb), which was calculated using the following Equation (2):

$$\text{MetMb (\%)} = [1.395 - ((A_{572} - A_{700}) / (A_{525} - A_{700}))] \times 100 \quad (2)$$

*where A_{525} , A_{572} , and A_{700} represent the absorbance values measured at 525 nm, 572 nm, and 700 nm, respectively.

2.11. Textural properties

The textural properties of the cooked meat batters were evaluated

using a Texture Analyzer (Texture Exponent 32, Stable Micro System, UK) equipped with a 25 mm aluminum cylindrical probe (model P/25 R) and a 50 kg load cell. Prior to texture analysis, the meat batter samples were cooked in a water bath at 75 °C for 30 min and then allowed to cool to room temperature. Texture measurements were performed on four different cuts (1 × 2 × 2 cm) obtained from the same batter group. The pre-test speed was set at 1 mm/s, while both the test and post-test speeds were set at 5 mm/s. Hardness, springiness, cohesiveness, and chewiness were calculated from the force–time curves obtained by double-compressing the samples at a 75 % compression ratio.

2.12. Microbiological analyses

Microbiological analyses were performed according to the method described by the FDA. (1998). For each group, 10 g of meat batter sample was aseptically placed into a sterile Stomacher bag and homogenized with 90 mL of Maximum Recovery Diluent (MRD) (Merck, Darmstadt, Germany). Serial dilutions were prepared and plated onto appropriate selective media using the pour-plate technique. Total aerobic mesophilic bacteria (TAMB) were enumerated on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) after incubation at 28 °C for 48 h. Total psychrophilic bacteria (TPB) were determined on the same medium following incubation at 6.5 °C for 10 days. Total coliforms were enumerated on Violet Red Bile Agar (VRBA) (Merck, Darmstadt, Germany) after incubation at 37 °C for 24 h. Total mold and yeast counts were assessed on Yeast Glucose Chloramphenicol Agar (YGCA) (Merck, Darmstadt, Germany) after incubation at 25 °C for 5 days. The microbial quality of GPP was evaluated using the same microbiological analysis procedures, including enumeration of total aerobic mesophilic bacteria, coliforms, and molds and yeasts.

According to the Turkish Food Codex Microbiological Criteria Regulation, *Salmonella* spp. and *Escherichia coli* O157 are not permitted in raw meat products (Turkish Food Codex Regulation on Microbiological Criteria, 2025). Therefore, all meat batter samples were tested for these pathogens. *Salmonella* analysis was performed using Buffered Peptone Water (BPW) for pre-enrichment, followed by selective enrichment in Rappaport-Vassiliadis Soya Broth (RVS) and Müller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn). For isolation, Xylose Lysine Deoxycholate (XLD) Agar and Brilliant Green Agar (BGA) were used. *E. coli* O157 was enriched in modified Tryptone Soya Broth (mTSB) supplemented with novobiocin and selectively isolated on Sorbitol MacConkey Agar (SMAC). Results were expressed as log₁₀ CFU/g.

2.13. Statistical analysis

All experiments were conducted in duplicate, and the results were reported as mean ± standard error. The effects of treatment (CON, BHT, GPP 0.5, GPP 0.75, GPP 1.0) and storage time (days 0, 2, 4, and 6) were evaluated using two-way analysis of variance (ANOVA) based on a randomized complete block design with a generalized linear model, performed using Minitab statistical software (Version 22.1, Minitab Inc., Enterprise Drive, State College, PA, USA). When the interaction between treatment and storage time was not significant, one-way ANOVA was used to evaluate the main effects of treatment or storage time. Statistically significant effects were further analyzed, and the means were compared using Tukey's test ($p \leq 0.05$).

3. Results and discussion

3.1. The characterization and phenolic profile of GPP

Table 1 presents the chemical composition and antioxidant properties of GPP from Kösetevék grapes. GPP was found to be rich in crude fiber (44.10 g/100 g dry GPP) and carbohydrates (27.37 g/100 g dry GPP), while protein, lipid, ash, and moisture levels were relatively low. These results align with previous findings by Cilli et al. (2020) and by

Table 1
Characterization and phenolic compounds identified in GPP (n = 3).

Parameters	Mean ± SEM
<i>Proximate composition(g/100 g dry GPP)</i>	
Moisture	6.70 ± 0.35
Protein	11.76 ± 1.12
Lipids	6.49 ± 0.09
Ash	3.58 ± 0.67
Crude fiber	44.10 ± 0.13
Carbohydrate	27.37 ± 0.05
<i>Physical–chemical parameters</i>	
Titratable acidity (g citric acid equivalents/100 g dry GPP)	5.28 ± 0.51
pH	3.58 ± 0.09
a _w	0.256 ± 0.06
<i>Phenolic compounds, and the antioxidant potential</i>	
Total phenolic (mg.GAE/100 g dry GPP)	191.11 ± 0.15
Total flavonoids (mg.CE/100 g dry GPP)	1.09 ± 0.09
DPPH (EC ₅₀ in µg/g dry GPP)	22.02 ± 3.13
<i>Color parameters</i>	
Lightness (L [*])	38.27 ± 0.19
Redness (a [*])	10.23 ± 0.07
Yellowness (b [*])	2.16 ± 0.25
Chroma (C [*])	10.45 ± 0.06
Hue (h [°])	11.93 ± 0.05
<i>Microbial counts (log CFU g)</i>	
Mesophilic bacteria	1.2 ± 0.95
Molds and yeasts	<1
Coliform bacteria	<1
<i>Phenolic compounds (µg/g dry GPP)</i>	
Gallic acid	325.20 ± 0.05
(+)-Catechin	343.20 ± 0.45
(-)-Epicatechin	205.60 ± 0.05
Luteolin-7-glucoside	243.20 ± 0.10
Resveratrol	44.40 ± 0.05
Protocatechuic acid	5.60 ± 0.02
Vanillin	4.80 ± 0.01
P-coumaric acid	10.40 ± 0.09
Taxifolin	22.00 ± 0.02
Rutin	6.80 ± 0.01
Ellagic Acid	11.20 ± 0.05

^a Data shown are the means ± standard error of the mean.

Andrés et al. (2017) for grape pomace.

To further characterize the functional potential of GPP, its pH value was determined as 3.58, which aligns with the pH values of various grape varieties (Cilli et al., 2020; Garrido et al., 2011). This low pH not only contributes to microbial stability but also plays a critical role in preserving anthocyanin color stability, as the characteristic red pigment tends to degrade in environments with pH above 4.5 (Enaru et al., 2021). The chemical structure of anthocyanins varies depending on the acidity or alkalinity of the food matrix, directly affecting the product's color quality. As expected, due to the presence of anthocyanins, the a* and b* color parameters of GPP were positive, while the L* value and hue angle (h°) were low, indicating a dark red pigmentation.

Regarding bioactive properties, the total phenolic content of the grape pomace powder was measured as 191.11 ± 0.15 mg GAE/100 g dry GPP, while the total flavonoid content was 1.09 ± 0.09 mg CE/100 g dry GPP. The DPPH radical scavenging assay revealed a strong antioxidant capacity with an EC₅₀ value of 22.02 ± 3.13 g dry GPP, supporting the high bioactive potential of GPP. The composition of grape pomace, and thus the profile of extractable compounds, is influenced by grape cultivar, ripening stage, environmental conditions, soil type, and processing techniques.

It was determined that the results obtained in this study differed from the total phenolic content (TPC) and total flavonoid content (TFC) values reported for various grape varieties in the literature. For example, in a study conducted on red and white grape varieties, TPC values ranged from 55.5 ± 0.87 to 153.8 ± 1.83 mg GAE/g extract, while TFC values ranged from 32.8 ± 0.41 to 91.7 ± 1.00 mg CE/g extract (Xu et al., 2016). Nakov et al. (2020) reported a total phenolic acid content of 126.1 ± 10.9 mg/kg and a total flavonoid content of 1461.5 ± 66.2

mg/kg in grape pomace powder. In another study, [Rodrigues et al. \(2023\)](#) reported TPC values ranging from 38.70 to 45.18 mg GAE/g and TFC values ranging from 14.94 to 18.29 mg CE/g in dried grape pomace using different solvent systems. However, due to differences in grape variety, extraction methods, units of expression, and analytical conditions, these values are not directly comparable to those obtained in the present study. The dominant presence of flavonoids highlights the potential of Kösetevек grape pomace as a functional food ingredient and suggests its value as a natural antioxidant source in the food industry.

According to microbiological analyses, total aerobic mesophilic bacteria in GPP were measured at 1.20 log CFU/g, while yeast, mold, and coliform bacteria were below 1 log CFU/g. These results demonstrate that GPP is a microbiologically safe, and its low moisture and water activity values may contribute to extending shelf life by slowing microbial, enzymatic, and non-enzymatic spoilage.

LC-MS/MS analysis identified a total of 12 phenolic compounds in GPP ([Table 1](#)). The most abundant compounds were catechin (343.20 µg/g), gallic acid (325.20 µg/g), luteolin-7-glucoside (243.20 µg/g), epicatechin (205.60 µg/g), and resveratrol (44.40 µg/g). Other phenolic compounds, including taxifolin, p-coumaric acid, rutin, vanillin, protocatechuic acid, and ellagic acid, were present in smaller amounts. However, compounds such as quercetin, ferulic acid, chlorogenic acid, naringenin, apigenin, and several others were not detected. Among the detected flavonoids, the presence of taxifolin and luteolin-7-glucoside in

Kösetevек GPP is particularly noteworthy. These compounds have not been previously reported in wine matrices, emphasizing the unique phenolic fingerprint of this grape variety. Taxifolin (3,5,7,3',4'-penta-hydroxyflavanone), commonly found in citrus fruits, onions, douglas fir bark, and larch, exhibits strong antioxidant, antimicrobial, antiviral, and anticancer properties, largely attributed to its dihydroflavonol structure ([Jain & Vaidya, 2023](#); [Sunil & Xu, 2019](#); [Topal et al., 2016](#)). Luteolin-7-glucoside, a glycosylated flavone, is widely found in vegetables and herbs such as celery, artichoke, and parsley, and is recognized for its anti-inflammatory and neuroprotective effects ([Caporali et al., 2022](#)).

The detection of these two flavonoids for the first time in grape pomace highlights the nutraceutical potential of Kösetevек pomace and represents a novel contribution to the understanding of phenolic profiles in less-studied grape varieties. Furthermore, the phenolic profile of Kösetevек GPP is predominantly composed of flavonoid compounds, especially flavanol derivatives such as (+)-catechin, (–)-epicatechin, luteolin-7-glucoside, and taxifolin, which collectively contribute to its potent antioxidant capacity. Gallic acid, a hydroxybenzoic acid derivative, also significantly enhances the extract's antioxidant potential ([Chedea et al., 2025](#)). These results are in line with previous studies that reported high levels of catechin and epicatechin in grape by-products. For instance, [Silva et al. \(2020\)](#) noted that drying methods influence their concentration in grape skins. [Milinčić et al. \(2021\)](#) identified

Table 2

Changes in moisture contents, weight loss, and pH values in beef model systems during storage at 4 °C (n = 4).

Variable	Interaction Treatment × time	Days of storage at 4°C				
		Day 0	Day 2	Day 4	Day 6	
Moisture (%)	CON	57.90 ± 0.02 ^{aE}	57.11 ± 0.10 ^{bE}	56.67 ± 0.12 ^{bE}	55.11 ± 0.13 ^{cE}	
	BHT	58.89 ± 0.02 ^{aD}	58.55 ± 0.04 ^{abD}	58.08 ± 0.03 ^{bcD}	57.38 ± 0.03 ^{cD}	
	GPP 0.5	61.78 ± 0.03 ^{aC}	60.60 ± 0.15 ^{bc}	59.92 ± 0.13 ^{bc}	59.15 ± 0.15 ^{cC}	
	GPP 0.75	65.36 ± 0.04 ^{aA}	64.66 ± 0.05 ^{aA}	63.87 ± 0.03 ^{bA}	62.97 ± 0.15 ^{cA}	
	GPP 1.0	63.66 ± 0.15 ^{abB}	63.31 ± 0.15 ^{abB}	62.82 ± 0.28 ^{bcB}	62.21 ± 0.34 ^{cB}	
	pH	CON	5.89 ± 0.09	5.93 ± 0.09	6.20 ± 0.03	6.91 ± 0.14
BHT		5.81 ± 0.13	5.90 ± 0.01	6.21 ± 0.23	6.82 ± 0.30	
GPP 0.5		5.68 ± 0.18	5.70 ± 0.01	5.98 ± 0.08	6.43 ± 0.22	
GPP 0.75		5.56 ± 0.11	5.52 ± 0.01	5.87 ± 0.03	6.66 ± 0.11	
GPP 1.0		5.49 ± 0.05	5.42 ± 0.18	5.85 ± 0.02	6.29 ± 0.19	
Weight loss (%)		CON	–	1.91 ± 2.11	1.38 ± 0.15	1.25 ± 1.27
	BHT	–	1.59 ± 0.98	1.26 ± 0.16	1.21 ± 0.27	
	GPP 0.5	–	1.51 ± 0.92	1.35 ± 1.51	1.29 ± 0.31	
	GPP 0.75	–	1.50 ± 1.31	1.26 ± 0.06	1.24 ± 0.63	
	GPP 1.0	–	1.51 ± 0.71	1.29 ± 0.12	1.26 ± 1.02	
	Main effects					
	Storage time	Day 0	Day 2	Day 4	Day 6	
Moisture (%)		61.52 ± 0.94	60.84 ± 0.94	60.27 ± 0.91	59.36 ± 0.98	
pH		5.68 ± 0.63 ^c	5.69 ± 0.70 ^c	6.02 ± 0.06 ^b	6.62 ± 0.10 ^a	
Weight loss		–	1.60 ± 0.98	1.31 ± 0.75	1.25 ± 0.61	
	Treatment	CON	BHT	GPP 0.5	GPP 0.75	GPP 1.0
Moisture (%)		58.22 ± 0.22	56.69 ± 0.39	60.36 ± 0.37	64.21 ± 0.34	62.99 ± 0.23
pH		6.18 ± 0.17 ¹	6.23 ± 0.16 ¹	5.95 ± 0.13 ²	5.90 ± 0.18 ²	5.76 ± 0.14 ²
Weight loss		1.51 ± 0.65	1.35 ± 0.31	1.38 ± 0.29	1.33 ± 0.94	1.35 ± 0.59
	P value	Treatment × time	Storage time	Treatment		
Moisture (%)		p < 0.001	p < 0.001	p < 0.001		
pH		ns	p < 0.01	p < 0.001		
Weight loss		ns	ns	ns		

* Data shown are the means ± standard error of the mean.

*ns: not significant.

*CON: Batter without any antioxidant (control sample), BHT: Batter containing synthetic antioxidant (BHT), GPP0.5: Batter containing 0.5 % grape pomace powder, GPP 0.75: Batter containing 0.75 % grape pomace powder, GPP 1.0: Batter containing 1.0 % grape pomace powder.

*^{a-c}: Means in the same row followed by different lowercase letters are significantly different due to the effect of storage time (p < 0.01); ^{A-E}: Means in the same column followed by different uppercase letters are significantly different due to the effect of treatment (p < 0.001); ^{1,2}: Means in the same row followed by different numbers are significantly different due to the effect of treatment (p < 0.001).

Note: When the interaction between treatment and storage time is statistically significant (p < 0.05), letter groupings are shown under the “Interaction” section; when only main effects are significant, groupings are presented under the “Main effects” section. No letter annotations are applied when no significant differences are observed.

seventy-five phenolic compounds in grape pomace, with ethyl gallate among the most abundant. Other studies have also confirmed that flavanol content varies depending on grape variety, processing, and extraction methods (Antoniolli et al., 2015; Baiano & Terracone, 2011; Ferreyra et al., 2020).

3.2. Moisture content, weight loss, and pH

Table 2 presents the moisture, pH, and weight loss values of each meat batter formulation during refrigerated storage. According to the ANOVA analysis, the interaction effect of “treatment × storage time” on moisture content was found to be statistically significant ($p < 0.001$). Overall, the moisture content of all samples decreased significantly throughout storage ($p < 0.001$). Among the formulations, the control and BHT groups exhibited the lowest moisture contents, while samples containing GPP maintained higher moisture levels. Notably, the GPP 0.75 and GPP 1.0 groups had the highest moisture contents at all storage points. Similarly, previous studies involving dietary fibers such as lemon, peach fiber, and adzuki bean flour have also reported increases in the moisture content of meat products (Aslinah et al., 2018; Soncu et al., 2015).

For pH values, both the main effects of “treatment” and “storage time” were statistically significant ($p < 0.01$ and $p < 0.001$, respectively). pH values increased significantly in all groups throughout storage, with slightly lower values observed in GPP-supplemented samples

compared to the control. By day 6, the highest pH values were recorded in the CON and BHT groups, measured at 6.91 and 6.82, respectively ($p > 0.001$), whereas the GPP-added groups showed a more limited increase. Among all formulations, the lowest pH value was detected in the GPP1.0 meat batter group ($p < 0.001$). Similarly, Abdelhakam et al. (2019) reported that the lowest pH value in beef burgers was found in the group containing grape pomace. This finding suggests that formulations enriched with GPP may have the potential to inhibit microbial activity and delay the rise in pH during storage. Furthermore, the increase in pH over time may be attributed to microbial protein degradation and the subsequent formation of amines, which are associated with spoilage in meat products. The literature also documents that an increase in microbial load during storage is accompanied by an increase in pH values (Abdelhakam et al., 2019).

In addition to these physicochemical changes, weight loss in meat batters ranged from 1.21 % to 1.91 %, and the addition of GPP or BHT did not cause any statistically significant differences in this parameter ($p > 0.05$). This lack of significant difference in weight loss is consistent with the trend of decreasing moisture content observed across all samples during storage. Although moisture content remained higher in GPP-added groups, weight loss was limited. This suggests that ingredients rich in dietary fiber, such as grape pomace, can enhance water-holding capacity and thereby help reduce weight loss during storage (Naghdi et al., 2025).

Table 3
Color parameters in beef model systems during storage at 4 °C (n = 8).

Variables	Interaction	Days of storage at 4°C				
	Treatment × time	Day 0	Day 2	Day 4	Day 6	
L*	CON	60.74 ± 1.71	59.66 ± 1.74	59.02 ± 1.33	56.58 ± 1.54	
	BHT	60.91 ± 0.29	59.41 ± 0.40	56.15 ± 0.08	55.22 ± 0.36	
	GPP 0.5	54.78 ± 1.15	53.66 ± 2.65	52.63 ± 1.89	50.87 ± 0.24	
	GPP 0.75	50.00 ± 1.95	49.34 ± 1.46	48.84 ± 1.65	48.03 ± 1.44	
	GPP 1.0	50.23 ± 2.16	49.44 ± 0.41	48.83 ± 0.65	47.17 ± 2.37	
a*	CON	19.36 ± 2.81	14.20 ± 1.90	11.51 ± 0.84	10.96 ± 0.87	
	BHT	19.24 ± 2.72	13.73 ± 1.07	13.43 ± 0.65	10.63 ± 0.59	
	GPP 0.5	15.48 ± 1.08	11.85 ± 0.79	10.05 ± 0.79	10.23 ± 0.41	
	GPP 0.75	14.25 ± 0.14	11.63 ± 0.46	10.42 ± 0.57	9.56 ± 0.03	
	GPP 1.0	12.95 ± 0.06	9.37 ± 0.73	9.53 ± 0.90	8.61 ± 0.68	
b*	CON	14.66 ± 0.17	13.35 ± 0.44	13.11 ± 0.07	11.94 ± 0.82	
	BHT	14.96 ± 0.31	13.54 ± 1.22	13.45 ± 0.40	10.85 ± 0.28	
	GPP 0.5	9.90 ± 0.18	7.93 ± 0.89	7.39 ± 0.90	6.58 ± 0.06	
	GPP 0.75	7.64 ± 0.38	6.39 ± 1.25	6.67 ± 0.85	6.32 ± 0.07	
	GPP 1.0	6.71 ± 0.18	5.44 ± 0.06	5.27 ± 0.02	5.11 ± 0.57	
	Main effects					
	Storage time	Day 0	Day 2	Day 4	Day 6	
L*		55.33 ± 1.69	54.30 ± 1.64	53.09 ± 1.45	51.57 ± 1.34	
a*		16.26 ± 1.06 ^A	12.16 ± 0.81 ^B	10.99 ± 0.72 ^B	9.99 ± 0.34 ^B	
b*		10.77 ± 1.16 ^A	9.30 ± 1.20 ^{AB}	9.15 ± 1.17 ^{AB}	8.23 ± 0.91 ^B	
	Treatment	CON	BHT	GPP 0.5	GPP 0.75	GPP 1.0
L*		59.00 ± 0.83 ^a	57.92 ± 0.89 ^a	52.98 ± 0.85 ^b	49.05 ± 0.68 ^{bc}	48.91 ± 0.98 ^c
a*		14.01 ± 1.43 ^{ab}	14.26 ± 1.52 ^a	11.90 ± 0.93 ^{bc}	11.47 ± 0.68 ^c	10.12 ± 0.68 ^c
b*		13.26 ± 0.41 ^a	13.20 ± 0.66 ^a	7.95 ± 0.53 ^b	6.76 ± 0.36 ^{bc}	5.63 ± 0.26 ^c
	P value	Treatment × time	Storage time	Treatment		
L*		ns	ns	$p < 0.001$		
a*		ns	$p < 0.001$	$p < 0.001$		
b*		ns	$p < 0.01$	$p < 0.001$		

* Data shown are the means ± standard error of the mean.

*ns: not significant.

*CON: Batter without any antioxidant (control sample), BHT: Batter containing synthetic antioxidant (BHT), GPP0.5: Batter containing 0.5 % grape pomace powder, GPP 0.75: Batter containing 0.75 % grape pomace powder, GPP 1.0: Batter containing 1.0 % grape pomace powder.

^{a, b, c}: Values within the same row with no common superscript differ significantly due to the effect of treatment ($p < 0.001$); A, B, C: Values within the same row with no common superscript differ significantly due to the effect of storage time ($p < 0.001$).

Note: When the interaction between treatment and storage time is statistically significant ($p < 0.05$), letter groupings are shown under the “Interaction” section; when only main effects are significant, groupings are presented under the “Main effects” section. No letter annotations are applied when no significant differences are observed.

3.3. Color coordinates

Table 3 presents the changes in color values of meat batter formulations during storage. Lightness (L^*) was significantly affected by “treatments” ($p < 0.001$), but no significant changes were observed over “storage time” ($p > 0.05$). The CON and BHT groups showed the highest L^* values, indicating a lighter surface color. In contrast, samples containing 0.75 % and 1.0 % GPP had significantly lower L^* values, suggesting a darker appearance. This effect is likely due to the phenolic content of GPP and its antioxidant activity, which contributes to color stabilization by inhibiting pigment oxidation (Gai et al., 2015; Sánchez-Alonso & Borderías, 2008). Phenolic compounds are known to scavenge reactive oxygen species (ROS) and chelate pro-oxidant metal ions (e.g., Fe^{2+} , Fe^{3+}), thereby preventing the oxidation of heme pigments such as myoglobin (Efenberger-Szmechtyk et al., 2021). In its reduced form, myoglobin imparts a desirable red color to meat, whereas oxidation leads to the formation of brown-colored metmyoglobin (Zhu et al., 2024). Thus, the phenolic constituents of GPP may help maintain meat color by delaying this oxidative transformation. Similar results have been reported in other studies using wine and grape pomace (de Alencar et al., 2022). This effect was explained by the presence of non-meat ingredients and the dilution of meat pigments. In another study, the addition of grape pomace was found to reduce the lightness of pork sausages, which was attributed to the presence of the 3-monoglucosidase enzyme in grape pomace (Ryu et al., 2014).

The a^* and b^* parameters were significantly affected by both “storage time” and “treatment” factors ($p < 0.01$ and $p < 0.001$, respectively); however, the interaction between these two factors was not significant ($p > 0.001$). Redness (a^*) values gradually decreased throughout the storage period, with the highest a^* values observed in the BHT group and the lowest in the GPP 1.0 group ($p < 0.001$). In terms of yellowness (b^*), the control and BHT groups had significantly higher b^* values compared to the GPP groups ($p < 0.001$). This situation may be attributed to possible causes such as lipid oxidation, myoglobin oxidation, and the oxidation of phenolic compounds present in grape pomace (Nerín et al., 2006). Such oxidative processes can alter pigment stability, leading to noticeable changes in product color (Luo et al., 2025). The color changes in L^* , a^* , and b^* values detected in this study were consistent with those reported by previous researchers (Andrés et al., 2017; Pereira et al., 2022; Riazzi et al., 2016).

3.4. Metmyoglobin

The color of meat, which depends on the chemical state of myoglobin, is an important factor influencing consumer acceptance. In fact, undesirable color changes occurring during storage are largely attributed to myoglobin oxidation and the formation of MetMb (Lee et al., 2017). Changes in MetMb content in beef model systems stored at 4 °C are presented in Fig. 1.

Both “storage time” and “treatment” significantly affected MetMb values ($p < 0.001$), and a gradual increase was observed by the end of day 6. This rise reflects the oxidation of deoxymyoglobin and oxymyoglobin to MetMb (Wu et al., 2024). MetMb levels were found to be significantly lower in the GPP-added groups compared to the control (CON) group ($p < 0.001$). This result indicates that GPP, particularly at higher concentrations, effectively delayed myoglobin oxidation during storage, as evidenced by consistently lower MetMb values in GPP-treated samples across all time points. The lower MetMb levels in GPP-added groups can be attributed to the ability of phenolic compounds to inhibit myoglobin oxidation by scavenging reactive oxygen species (ROS) and chelating pro-oxidant metal ions. Previous studies have shown that phenolic compounds can inhibit MetMb formation during storage by preventing myoglobin oxidation (Han et al., 2024; Inai et al., 2014). On day 2 of storage, a significant reduction in MetMb levels was observed across all groups. This decrease can be attributed to the natural metmyoglobin reducing activity (MRA) of the meat and the free

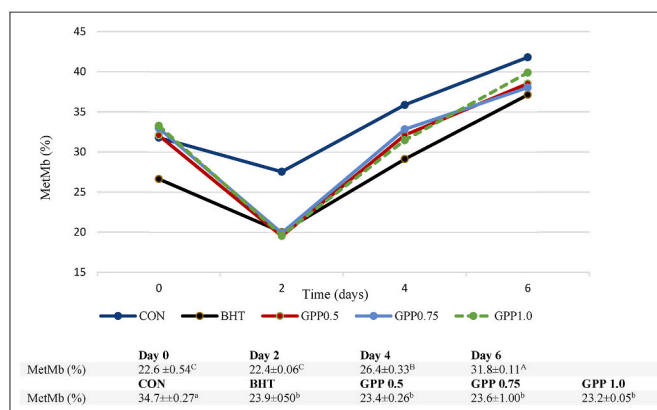


Fig. 1. Changes in MetMb values of beef model systems during storage at 4 °C ($n = 4$)

*Values in the same row marked with different uppercase letters (A–C) are significantly different due to the effect of storage time ($p < 0.001$).

Values in the same row marked with different lowercase letters (a–c) are significantly different due to the effect of treatment ($p < 0.001$).

radical scavenging effects of the antioxidant treatments (BHT and GPP) (Hoa et al., 2021; Pujol et al., 2023; Wang et al., 2021). At this stage, MetMb levels dropped to approximately 19–20 % in both GPP and BHT-treated groups. In contrast, MetMb accumulation in the CON group progressed more rapidly, reaching 41.8 % by day 6. MetMb levels in the GPP-added groups were significantly lower than those in the CON group ($p < 0.001$), indicating that antioxidant supplementation helped delay MetMb formation and preserve color stability.

Although oxidative changes became more dominant as storage time progressed, GPP and BHT treatments generally delayed browning compared to the CON group. No significant differences were detected among the BHT and various GPP treatment groups, indicating that all antioxidant treatments provided a comparable level of protection against color deterioration. These findings are consistent Muño et al. (2014), who reported that 200 mg GAE/kg grape pomace extract effectively delayed MetMb accumulation and preserved meat color within acceptable limits. Similarly, Mtibaa et al. (2019) demonstrated that onion peel extract delayed MetMb formation and maintained color stability in ground beef. Bao et al. (2008) also found that natural antioxidants like ergothioneine supported meat color by preventing lipid and protein oxidation.

3.5. TBARS

Fig. 2 presents the changes in TBARS values in beef model systems during storage at 4 °C. According to the ANOVA results, both “treatment” and “storage time” had a statistically significant effect on TBARS values ($p < 0.001$). The highest TBARS values were observed in the CON group, followed by the BHT group, while the lowest values were recorded in the GPP-added groups ($p < 0.001$). These results indicate that GPP significantly reduced lipid oxidation and may exhibit antioxidant potential comparable to that of BHT. Notably, lipid oxidation levels were lowest on day 0 in the samples treated with GPP, which can be attributed to the antioxidant effect of grape polyphenols that stabilize meat quality from the onset of storage. Supporting this observation, Banon et al. (2007) reported similarly low TBARS values on day 0 in raw beef patties supplemented with green tea and grape seed extracts. Likewise, Garrido et al. (2011) demonstrated that the addition of grape extract effectively reduced lipid oxidation in pork burgers on the first day of storage.

A comparison among the GPP groups showed similar TBARS values at 0.5 %, 0.75 %, and 1.0 % concentrations ($p > 0.05$). In particular, the mean TBARS value for the GPP 1.0 group (0.52 mg MDA/kg) was found

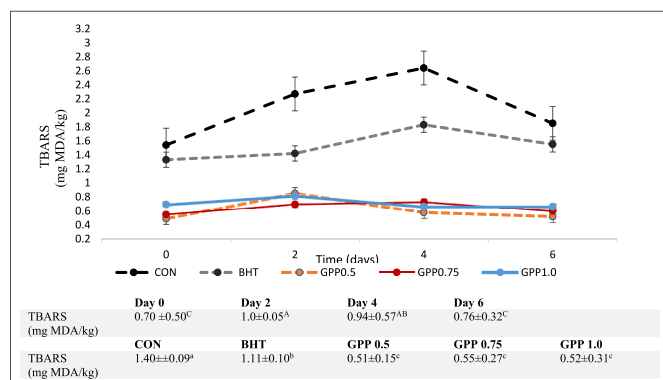


Fig. 2. Changes in TBARS values of beef model systems during storage at 4 °C (n = 4)

*Values marked with different uppercase letters (A–C) in the same row indicate significant differences due to the effect of storage time ($p < 0.001$).

Values marked with different lowercase letters (a–c) in the same row indicate significant differences due to the effect of treatment ($p < 0.001$).

to be quite similar to those of the lower concentration groups, GPP 0.5 and GPP 0.75. This suggests that increasing the amount of GPP does not necessarily have a concentration-dependent effect on reducing lipid oxidation. Essentially, the antioxidant effect of grape pomace is attributed to the ability of its phenolic compounds to scavenge free radicals, form complexes with metal ions, and inhibit or reduce the formation of singlet oxygen species (Milincić et al., 2021; Moro et al., 2021). However, the antioxidant activity of phenolic compounds can vary depending on several factors, including their chemical structure, interactions with the food matrix, the presence of metal ions, as well as their stability and bioavailability (Bai et al., 2025; Lopes et al., 2025). Supporting this, Cilli et al. (2020) reported that GPP reduced TBARS values (0.18–0.36 mg MDA/kg) in salmon burgers comparably to BHT. Similarly, Riaz et al. (2016) demonstrated that 1 % grape pomace combined with reduced nitrite lowered TBARS levels and delayed lipid oxidation in beef sausages.

In all samples, except the CON group, a decreasing trend in TBARS levels was observed starting from day 4 ($p < 0.001$). A gradual reduction was noted in both the group containing the synthetic antioxidant (BHT) and the samples with GPP addition (Fig. 2). As stated in the literature, antioxidants have been shown to slow oxidative processes by forming stable complexes with myoglobin, thereby reducing the availability of iron (Suman & Joseph, 2013). In addition, the decrease in TBARS values may be attributed to the degradation of MDA by certain microorganisms. Moreover, existing MDA may react with thiobarbituric acid to form other compounds such as alcohols and acids, leading to a reduction in measurable MDA levels (Riaz et al., 2016).

Over time, the decrease in TBARS values may also be attributed to the instability of MDA under certain storage conditions. MDA is a highly reactive and unstable compound that can undergo polymerization or react with proteins and other components of the meat matrix during prolonged storage, leading to the formation of new compounds. These reactions can lead to the formation of non-volatile secondary products that are not detectable by the TBARS assay, resulting in lower measured MDA levels (Abeyrathne et al., 2021). Overall, these findings highlight the potential of grape pomace powder as an effective natural antioxidant that can inhibit lipid oxidation in meat products, providing an alternative to synthetic antioxidants like BHT.

3.6. Textural properties

The findings of this study demonstrate that, in meat model systems with different formulations, all measured textural parameters except cohesiveness were significantly affected by storage time ($p < 0.01$ and $p < 0.001$, Table 4). Moreover, in GPP-formulated samples, cohesiveness,

gumminess, and chewiness showed significant differences compared to the CON group ($p < 0.001$). The reduction in hardness observed during storage is mainly attributed to the gradual loss of water-holding capacity over time, as hardness in meat systems is closely related to the structure of the protein matrix and its ability to retain moisture (Melro et al., 2020). However, this decrease was not statistically significant ($p > 0.05$), indicating that although hardness was better preserved in GPP-containing groups, the difference was not substantial enough to be considered significant. This outcome may be attributed to the high dietary fiber content of GPP, particularly cellulose and lignin, which are known to strengthen the protein matrix and slow moisture migration (M. Han & Bertram, 2017).

Based on the data presented in Table 4, an increasing level of GPP in the formulations was associated with a decreasing trend in cohesiveness and gumminess values. However, statistical differences in cohesiveness were observed only between the CON and the GPP 1.0 group ($p < 0.05$), suggesting a dose-dependent effect. Notably, chewiness was highest in the GPP1.0 group, compared to the CON and BHT groups ($p < 0.001$). A similar result was reported by de Alencar et al. (2022), who found that the addition of 0.5–2.0 g of grape pomace to beef burgers significantly increased chewiness. Throughout storage, cohesiveness, gumminess, and chewiness values tended to decrease across all groups ($p < 0.01$; $p < 0.001$), likely due to a loss of protein functionality, which leads to reduced structural integrity and, consequently, lower cohesiveness.

3.7. Microbiological analysis

Changes in total aerobic microorganisms (mesophilic and psychrotrophic bacteria), total coliforms (TCC), and yeast and mold counts (log CFU/g) in beef model systems over 6 days of storage at 4 °C are presented in Table 5. Pathogen analyses for *Salmonella* spp. and *Escherichia coli* O157 were conducted on all meat batters during storage, and neither pathogen was detected in any sample. All microbial groups showed a statistically significant increase during storage ($p < 0.001$). Initial counts ranged from 3.42 to 3.47 log CFU/g for mesophiles and 3.08–3.24 log CFU/g for psychrotrophs. By day 6, the highest microbial loads were observed in the CON and BHT groups, while GPP-supplemented groups showed relatively lower increases ($p < 0.001$). These findings align with previous studies on lamb patties containing grape pomace, which reported similar total aerobic counts after 7 days of storage (Andrés et al., 2017). TCC and yeast and mold counts also increased significantly ($p < 0.05$), reaching 6.29–6.70 log CFU/g and 6.42–6.65 log CFU/g, respectively, by the end of storage. Coliform growth was notably lower in GPP groups ($p < 0.001$). While mesophilic and psychrotrophic counts in GPP groups were significantly lower than those in CON and BHT groups at day 6, the effect of GPP on yeast and mold growth appeared limited. Although GPP supplementation reduced microbial proliferation to some extent, all samples exceeded the acceptable spoilage threshold (10^6 – 10^7 CFU/g) by day 6. This may be attributed to the low concentration of GPP used, as the antimicrobial efficacy of natural extracts often diminishes in complex food systems (Gai et al., 2015). These results are consistent with observations by Garrido et al. (2011) in pork burgers with grape extract. Overall, antioxidant addition alone was insufficient to fully prevent spoilage during chilled storage.

4. Conclusion

This study demonstrated that grape pomace powder (GPP) obtained from the local Kösetevék grape variety possesses strong antioxidant potential and moderate antimicrobial activity in beef model systems. GPP effectively delayed lipid oxidation and reduced metmyoglobin formation, thereby enhancing oxidative stability. Although microbial growth was significantly lower in GPP-treated groups compared to the control, spoilage levels still exceeded acceptable limits by the end of storage, indicating that higher concentrations or combined preservation

Table 4
Textural parameters in beef model systems during storage at 4 °C (n = 8).

Variables	Interaction	Days of storage at 4°C				
	Treatment × time	Day 0	Day 2	Day 4	Day 6	
Hardness (N)	CON	19794 ± 2.93	18104 ± 1.75	19589 ± 3.32	15612 ± 1.24	
	BHT	19138 ± 12.47	17285 ± 3.50	16191 ± 4.15	14510 ± 1.48	
	GPP 0.5	19853 ± 1.52	19103 ± 1.13	17793 ± 1.37	15066 ± 3.43	
	GPP 0.75	18976 ± 0.91	20116 ± 4.76	18405 ± 11.80	15599 ± 2.20	
	GPP 1.0	18428 ± 7.98	20476 ± 9.50	19301 ± 4.62	17663 ± 2.25	
Springiness (mm)	CON	0.90 ± 0.05	1.42 ± 0.58	1.74 ± 0.95	0.94 ± 1.22	
	BHT	0.87 ± 1.36	0.99 ± 0.16	1.60 ± 0.91	0.88 ± 0.06	
	GPP 0.5	0.90 ± 2.05	0.86 ± 1.36	1.54 ± 0.77	0.82 ± 0.09	
	GPP 0.75	0.86 ± 2.12	0.78 ± 0.11	1.84 ± 1.06	0.84 ± 3.03	
	GPP 1.0	0.77 ± 0.11	0.83 ± 1.15	1.72 ± 1.09	0.75 ± 1.69	
Cohesiveness (N mm)	CON	0.33 ± 0.03	0.28 ± 4.04	0.39 ± 1.04	0.32 ± 0.69	
	BHT	0.36 ± 1.01	0.26 ± 3.02	0.25 ± 0.05	0.31 ± 0.41	
	GPP 0.5	0.33 ± 0.11	0.29 ± 0.19	0.28 ± 2.01	0.29 ± 0.04	
	GPP 0.75	0.29 ± 2.04	0.26 ± 0.16	0.26 ± 1.03	0.26 ± 0.52	
	GPP 1.0	0.27 ± 1.48	0.25 ± 0.41	0.25 ± 0.01	0.24 ± 0.99	
Gumminess (N)	CON	7174 ± 2.23	5288 ± 4.49	5483 ± 1.93	5184.5 ± 1.40	
	BHT	6819 ± 2.26	5106 ± 0.91	5299.4 ± 0.68	3789 ± 1.51	
	GPP 0.5	6430 ± 2.60	5344.8 ± 4.05	4880 ± 1.89	3915 ± 0.86	
	GPP 0.75	6335 ± 1.79	5223.7 ± 0.95	4667 ± 4.91	3537 ± 2.01	
	GPP 1.0	5049.3 ± 3.72	4687.3 ± 7.52	4266 ± 1.04	33381 ± 4.32	
Chewiness (N mm)	CON	5060 ± 2.36	4688 ± 1.20	4722 ± 2.35	4713 ± 4.58	
	BHT	5500.8 ± 7.72	4946 ± 0.95	5199 ± 2.67	3561 ± 3.47	
	GPP 0.5	5691 ± 1.21	5064 ± 7.15	5652.2 ± 6.89	4332 ± 2.25	
	GPP 0.75	7854 ± 10.57	5177 ± 4.54	6015 ± 10.02	4505 ± 10.05	
	GPP 1.0	8060 ± 9.52	5468 ± 6.65	6073.9 ± 3.80	5682.4 ± 7.16	
Main effects						
	Storage time	Day 0	Day 2	Day 4	Day 6	
Hardness		19238 ± 3.89 ^a	19017 ± 5.33 ^a	18256 ± 8.91 ^a	15690 ± 5.67 ^b	
Springiness		0.86 ± 0.98 ^b	0.98 ± 1.20 ^{ab}	1.68 ± 0.33 ^a	0.85 ± 2.76 ^b	
Cohesiveness		0.30 ± 0.32	0.27 ± 0.78	0.28 ± 0.93	0.29 ± 0.12	
Gumminess		6361 ± 2.49 ^a	5130 ± 1.71 ^b	4919 ± 1.69 ^b	3961 ± 2.26 ^c	
Chewiness		6433 ± 4.73 ^a	5068 ± 1.76 ^{bc}	5532 ± 1.80 ^b	4559 ± 2.46 ^c	
	Treatment	CON	BHT	GPP 0.5	GPP 0.75	GPP 1.0
Hardness		18275 ± 9.79	16781 ± 1.76	17954 ± 7.83	18274 ± 7.78	18967 ± 4.62
Springiness		1.25 ± 0.25	1.09 ± 0.21	1.03 ± 1.18	1.08 ± 0.26	1.02 ± 1.25
Cohesiveness		0.34 ± 0.26 ^A	0.29 ± 0.85 ^{AB}	0.29 ± 1.21 ^{AB}	0.27 ± 0.15 ^{AB}	0.24 ± 0.08 ^B
Gumminess		5782 ± 3.23 ^A	5253 ± 4.44 ^{AB}	5142 ± 3.47 ^B	4941 ± 3.96 ^{BC}	4346 ± 2.50 ^C
Chewiness		4796 ± 1.24 ^C	4802 ± 2.94 ^C	5185 ± 2.53 ^{BC}	5888 ± 5.22 ^{AB}	6321 ± 4.41 ^A
	P value	Treatment × time	Storage time	Treatment		
Hardness		ns	p < 0.001	ns		
Springiness		ns	p < 0.01	ns		
Cohesiveness		ns	ns	p < 0.001		
Gumminess		ns	p < 0.001	p < 0.001		
Chewiness		ns	p < 0.001	p < 0.001		

*Data shown are the means ± standard error of the mean; ns: not significant.

*CON: Batter without any antioxidant (control sample), BHT: Batter containing synthetic antioxidant (BHT), GPP0.5: Batter containing 0.5 % grape pomace powder, GPP 0.75: Batter containing 0.75 % grape pomace powder, GPP 1.0: Batter containing 1.0 % grape pomace powder.

* A, B, C: Values within the same row with no common superscript differ significantly due to the effect of treatment (p < 0.001); a, b, c: Values within the same row with no common superscript differ significantly due to the effect of storage time (p < 0.001).

Note: When the interaction between treatment and storage time is statistically significant (p < 0.05), letter groupings are shown under the “Interaction” section; when only main effects are significant, groupings are presented under the “Main effects” section. No letter annotations are applied when no significant differences are observed.

strategies may be required for effective microbial control. In addition to well-known phenolic compounds such as gallic acid, (+)-catechin, and (–)-epicatechin, this study is, to the best of our knowledge, the first to report the presence of taxifolin and luteolin-7-glucoside flavonoids in the Kösetevék grape among grape varieties. This novel finding underscores the potential of the Kösetevék grape—with its distinctive phenolic profile—as a natural alternative to synthetic antioxidants in meat products.

Limitations of the study

This study was limited to the use of grape pomace powder derived from a single local grape variety (Kösetevék) and applied only to a

specific beef model system. The evaluation was restricted to three concentration levels (0.5 %, 0.75 %, and 1.0 %), and no sensory analysis or consumer acceptance testing was conducted. Therefore, the generalizability of the findings to other food systems or higher inclusion levels remains limited. In addition, the environmental and economic impacts of grape pomace utilization were not assessed in this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 5
Microbiological parameters in beef model systems during storage (n = 4).

Variable	Interaction				
	Treatment × time	Day 0	Day 2	Day 4	Day 6
Mesophilic bacteria	CON	3.47 ± 0.05	4.53 ± 0.02	5.62 ± 0.11	7.19 ± 0.08
	BHT	3.46 ± 0.04	4.55 ± 0.03	5.66 ± 0.01	7.26 ± 0.10
	GPP 0.5	3.42 ± 0.11	4.43 ± 0.05	5.77 ± 0.15	6.97 ± 0.03
	GPP 0.75	3.40 ± 0.01	4.44 ± 0.05	5.78 ± 0.16	6.98 ± 0.87
	GPP 1.0	3.42 ± 0.07	4.43 ± 0.15	5.60 ± 0.01	6.98 ± 0.04
Psychrotrophic bacteria	CON	3.24 ± 0.01	4.37 ± 0.02	5.40 ± 0.83	6.94 ± 0.08
	BHT	3.27 ± 0.03	4.39 ± 0.02	5.44 ± 0.05	7.02 ± 0.11
	GPP 0.5	3.17 ± 0.02	4.27 ± 0.15	5.35 ± 0.21	6.55 ± 0.09
	GPP 0.75	3.10 ± 0.09	4.20 ± 0.02	5.31 ± 0.19	6.40 ± 0.04
	GPP 1.0	3.08 ± 0.17	4.17 ± 0.04	5.27 ± 0.11	6.43 ± 0.14
Coliform bacteria	CON	2.97 ± 0.04	3.93 ± 0.19	5.37 ± 0.18	6.63 ± 0.08
	BHT	2.97 ± 0.16	3.99 ± 0.06	5.40 ± 0.07	6.70 ± 0.11
	GPP 0.5	2.76 ± 0.14	3.78 ± 0.01	5.32 ± 0.06	6.34 ± 0.18
	GPP 0.75	2.72 ± 0.09	3.68 ± 0.09	5.28 ± 0.08	6.30 ± 0.06
	GPP 1.0	2.60 ± 0.05	3.69 ± 0.08	5.24 ± 0.13	6.29 ± 0.63
Mold and Yeasts	CON	3.25 ± 0.06	4.13 ± 0.05	5.89 ± 0.10	6.65 ± 0.06
	BHT	2.97 ± 0.03	4.16 ± 0.78	5.89 ± 0.04	6.65 ± 0.11
	GPP 0.5	2.93 ± 0.05	4.00 ± 0.09	5.58 ± 0.28	6.41 ± 0.05
	GPP 0.75	2.91 ± 0.06	3.91 ± 0.05	5.39 ± 0.13	6.45 ± 0.12
	GPP 1.0	2.92 ± 0.01	3.85 ± 0.11	5.57 ± 0.87	6.42 ± 0.91
<i>E. coli</i> O157	CON	–	–	–	–
	BHT	–	–	–	–
	GPP 0.5	–	–	–	–
	GPP 0.75	–	–	–	–
	GPP 1.0	–	–	–	–
<i>Salmonella</i>	CON	–	–	–	–
	BHT	–	–	–	–
	GPP 0.5	–	–	–	–
	GPP 0.75	–	–	–	–
	GPP 1.0	–	–	–	–
	Main effects				
	Storage time	Day 0	Day 2	Day 4	Day 6
Mesophilic bacteria		3.43 ± 0.01 ^d	4.48 ± 0.02 ^c	5.68 ± 0.04 ^b	7.08 ± 0.05 ^a
Psychrotrophic bacteria		3.17 ± 0.03 ^d	4.28 ± 0.03 ^c	5.35 ± 0.03 ^b	6.67 ± 0.21 ^a
Coliform bacteria		2.81 ± 0.06 ^d	3.81 ± 0.04 ^c	5.32 ± 0.02 ^b	6.45 ± 0.07 ^a
Mold and Yeasts		2.97 ± 0.05 ^d	4.01 ± 0.04 ^d	5.65 ± 0.07 ^d	6.48 ± 0.05 ^d
	Treatment	Mesophilic bacteria	Psychrotrophic bacteria	Coliform bacteria	Mold and Yeasts
CON		5.21 ± 0.52 ^{AB}	4.99 ± 0.61 ^A	4.73 ± 0.53 ^A	4.98 ± 0.56 ^A
BHT		5.23 ± 0.53 ^A	5.03 ± 0.52 ^A	4.77 ± 0.53 ^A	4.89 ± 0.54 ^A
GPP 0.5		5.15 ± 0.51 ^{AB}	4.83 ± 0.48 ^B	4.55 ± 0.52 ^B	4.73 ± 0.51 ^B
GPP 0.75		5.14 ± 0.51 ^{AB}	4.75 ± 0.47 ^C	4.50 ± 0.52 ^B	4.64 ± 0.50 ^B
GPP 1.0		5.11 ± 0.50 ^B	4.74 ± 0.57 ^C	4.46 ± 0.53 ^B	4.64 ± 0.52 ^B
	P value	Treatment × time	Storage time	Treatment	
Mesophilic bacteria		ns	p < 0.001	p < 0.001	
Psychrotrophic bacteria		ns	p < 0.001	p < 0.001	
Coliform bacteria		ns	p < 0.001	p < 0.001	
Mold and Yeasts		ns	p < 0.001	ns	

* Data shown are the means ± standard error of the mean.

*ns: not significant.

*CON: Batter without any antioxidant (control sample), BHT: Batter containing synthetic antioxidant (BHT), GPP0.5: Batter containing 0.5 % grape pomace powder, GPP 0.75: Batter containing 0.75 % grape pomace powder, GPP 1.0: Batter containing 1.0 % grape pomace powder.

^{a, b, c}: Values within the same row with no common superscript differ significantly due to the effect of treatment (p < 0.001); A, B, C: Values within the same column with no common superscript differ significantly due to the effect of storage time (p < 0.001).

Note: When the interaction between treatment and storage time is statistically significant (p < 0.05), letter groupings are shown under the “Interaction” section; when only main effects are significant, groupings are presented under the “Main effects” section. No letter annotations are applied when no significant differences are observed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2025.118344>.

Data availability

Data will be made available on request.

References

- *These five key references provide the scientific basis for our study by demonstrating the antioxidant and quality-enhancing effects of grape pomace in meat products. They are directly comparable to our work in terms of raw material, product type, and quality parameters evaluated. Thus, they support the relevance and originality of our research.
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