

The Use of Secondary Grape Biomass in Beef Cattle Nutrition on Carcass Characteristics, Quality and Shelf Life of Meat

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How to cite this paper: Molosse, V.L., Deolindo, G.L., Lago, R.V.P., Klein, B., Zotti, C.A., Vedovato, M., da Silveira, M.V., Copetti, P.M., Schetinger, M.R.C., Favero, J.F., Fiorentin, E.L. and da Silva, A.S. (2024) The Use of Secondary Grape Biomass in Beef Cattle Nutrition on Carcass Characteristics, Quality and Shelf Life of Meat. *Food and Nutrition Sciences*, 15, 447-469.

<https://doi.org/10.4236/fns.2024.156030>

Received: April 8, 2024

Accepted: June 23, 2024

Published: June 26, 2024

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Abstract

We determined whether the inclusion of 100 g/kg dry matter of grape pomace silage (GPS) and grape pomace bran (GPB) as substitutes for other traditional fiber sources in the diet of steers (Charolais x Nellore) would improve carcass characteristics, meat quality and composition, and shelf life. Twenty-four animals (248 ± 19.32 kg of initial body weight) were fed a high concentrate diet for 121 days. Carcass characteristics were measured, and the longissimus dorsi muscle was analyzed for fatty acid (FA) profile and composition. The meat was sliced and stored in air-permeable packages for 10 days. On each sampling day (d 1, 3, 7, and 10), oxidative stability, bacterial load, lipid and protein oxidation, and staining were analyzed. The experimental diets influenced the pH of cold carcasses only. The GPS group had a higher pH than the control. The GPS and GPB groups showed improved oxidant status (i.e., lower lipid peroxidation and concentrations of reactive oxygen species were in the meat of both groups than in control). On the first day of storage, the antioxidant enzyme glutathione S-transferase activity was more significant in the meat of the GPS and GPB groups than in the control. The bacterial loads in the meat were attenuated by GPS inclusion; there were lower total coliform counts and a trend toward lower counts for enterobacteria in the control group. The diets altered the FA profile of the meat; i.e., the GPB diet allowed for a more significant amount of the n-6 omegas in the meat, while the GPS diet showed a tendency for a more significant amount of n-6 and 9

omegas. Both diets (GPS and GPB) increased the amounts of long-chain FAs. The GPS diet decreased saturated FA levels. We conclude that the dietary treatments GPS and GPB are a promising alternative to maintain meat quality standards throughout in real-world retail conditions. These treatments gave rise to an improvement in the nutritional value of the meat due to the more significant amounts of FAs that improve human health.

Keywords

Animal Nutrition, Antioxidant, Biomass, Grape, Meat Quality, Microbiology, Residue

1. Introduction

The shelf life and quality of beef are influenced by oxidative processes, microbiological growth, and color stability; these parameters are critical for consumer acceptance and to maximize shelf life. Retail conditions that expose meat to high oxygen concentrations potentiate oxidation [1], potentially harmful to health [2] [3].

To prolong shelf life, antioxidants are added to mitigate lipid oxidation, delay the development of unpleasant flavors, and improve color stability [4]. However, some studies found synthetic antioxidants to be toxic and carcinogenic [5]-[8]. Therefore, there is substantial demand for alternative antioxidants, which are standard in the food industry.

The use of secondary biomass obtained from post-harvest processing of grape crops in ruminant nutrition to sustain livestock farming; ruminants convert fibrous components into high-value proteins [9]. These residues contain substantial numbers of phenolic compounds with functional capabilities [10]. In animal nutrition, grapes in diets were linked to increased microbiological capacity [11] and oxidative stability [12]. For example, as a feed additive for weaned piglets there was a significant increase in the tissue/cellular antioxidant capacity [elevated glutathione (GSH) levels, higher H₂O₂ decomposition activities, and an enhanced overall antioxidant capacity] and decreased oxidative stresses. Furthermore, increased the growth of probiotic and lactic acid bacteria while inhibiting the growth of pathogenic bacterial species, including *C. jejuni* and the Enterobacteriaceae group in animal gut [13]. The total antioxidant capacity against peroxy radicals (superoxide dismutase and glutathione peroxidase activities) was higher in the serum of laying hens fed a diet based on grape pomace flour [14]. Furthermore, there were increased enterococcal populations and decreasing Clostridia counts were observed in the ileal content of grape-pomace-fed birds [15]. The addition of grape seed extract combined with vacuum-packaging significantly improved lipid oxidation stability (thiobarbituric acid-reactive substances (TBARS) and volatile compound formation) of minced turkey meat during heat treatment and during 13 days of refrigerated storage

[12]. The proanthocyanidins in grapes (i.e., condensed tannins) influence ruminal biohydrogenation [16], allowing the passage of polyunsaturated FAs (PUFAs) unchanged in the rumen to be absorbed distally, providing meat with better lipid composition and nutritional value [17].

Food costs have risen for various reasons, and farmers seek alternative ingredients, especially industrial wastes. Therefore, the objective of the present study was to determine whether the inclusion of 100 g kg⁻¹ of grape residue in ensiled and dehydrated form in the diets for feedlot steers (replacing other traditional fiber sources) would improve meat composition and quality during storage under retail conditions. Our hypothesis is that both residues (GPS and GPB) can improve meat quality aspects, by providing greater meat stability during shelf life. In particular, we believe that the GPB has greater use by the animals, since the residue received physical processing, and thus, more evident beneficial effects on meat quality, when compared to those receiving the raw residue (GPS).

2. Materials and Methods

2.1. Experiment Location

The Animal Research Ethics Committee of the Universidade do Estado de Santa Catarina approved the project (protocol number: 4948210322), following the guidelines of CONCEA/Brasil.

2.2. Preparation of Grape Pomace Silage (GPS) and Grape Pomace Brans (GPB)

The GPS in cv. Isabel (*Vitis labrusca*) is composed of 3.63% stems, 50.19% barks, and 46.18% seeds (based on the dry matter). This material was collected in the municipality of Pinheiro Preto (27° 03' 02" S; 51° 13' 51" W) at an industrial winery. The residue was derived from the pressing process and separation of the solid fraction. Soon after being collected, the GPS was transported for storage in a trench-type silo (3.0 × 0.9 × 6.0 m for width, height, and length, respectively), coated with plastic on the sides and bottom. Once the GPS was placed in the silo, it was compacted with an agricultural tractor and then the silo was sealed with specific tarpaulin for storage of preserved bulky foods (Parcifil[®], Sapiranga, Brazil). The GPS was ensiled for 3 months before starting the experiment, characterized as grape pomace silage (GPS).

The production of grape pomace bran (GPB) required dehydrating the GPS. For logistical reasons, it was necessary to dry the GPS in two batches. The first dehydrated batch was carried out in a closed environment, using gas heater and fans to heat the environment and force air circulation. The second batch of GPS was dehydrated in an open environment (i.e., in the sun) on plastic sheets. Both forms of drying were carried out at the Universidade do Estado de Santa Catarina (27° 06' 17" S 52° 36' 51" W) and lasted about 7 days until reaching values below 10% humidity to avoid deterioration during storage. After drying, the batches were mixed and ground using an industrial hammer grinder, using a sieve that provided a particle size of 4 mm.

2.3. Experimental Diets and Feed

Diets were formulated to meet nutritional requirements for an estimated average daily gain of 1.5 kg of body weight (BR-CORTE, 2016). By-products (GPS or GPB) were added to the diet based on total replacement of wheat bran and partial replacement of soybean hulls from the Control diet (Table 1). The rations for the three dietary treatments were isonitrogenous and isoenergetic.

Twenty-four steers were used, from industrial crosses ($\frac{1}{2}$ Charolais x $\frac{1}{2}$ Nellore), to constitute three dietary treatments (eight steers/treatment) in a randomized design to standardize initial body weight between groups, considering each steer as an experimental unit. The animals from each treatment were fed in individual pens: control diet (traditional feed diet), GPS diet (inclusion of 100 g kg^{-1} in the dry matter (DM) of grape pomace silage in the diet), or GPB diet (inclusion of 100 g kg^{-1} in the DM of grape pomace bran in the diet). The study lasted 121 days with 21 days of diet adaptation and 100 days of the experimental period. The production aspects were previously published by Molosse *et al.* [18].

Table 1. Ingredient and chemical composition of experimental diets.

Item	Ingredient (g kg^{-1})			Dietary Treatment		
	GPS	GPB	WB	Control	GPS	GPB
Grape pomace silage (GPS)				-	100.0	-
Grape pomace bran (GPB)				-	-	100.0
Corn Silage				400.0	400.0	400.0
Ground Corn				280.3	351.2	352.2
Soybean meal				29.1	48.2	47.5
Soybean hulls				139.8	58.7	59.1
Wheat bran (WB)				110.0	-	-
Minerals ¹				22.0	22.2	22.0
Urea				7.0	7.5	7.0
Sodium bicarbonate				10.0	10.0	10.0
Mycotoxin adsorbent				2.2	2.2	2.2
	Chemical composition (g kg^{-1})					
Dry matter (DM)	415.0	934.0	847.5	440.7	404.0	475.9
Organic matter (OM)	951.1	968.3	948.4	945.9	950.5	950.7
Ash	48.9	31.7	51.6	54.1	49.5	49.3
Ether extract	112.3	150.9		23.3	30.8	34.5
Crude protein	117.0	136.0	148.9	108.3	111.4	111.7
Neutral detergent fiber	620.0	586.0	436.5	381.4	387.2	350.7
Acid detergent fiber	594.0	535.0	139.2	221.0	244.7	218.6
Lignin	334.0	307.0	48.8	27.7	75.3	55.9
Starch	24.6	11.00	233.7	302.7	349.4	366.5

Continued

Total carbohydrates	721.8	681.4	799.5	814.3	805.4	804.5
Pectin + sugar	90.8	70.8	129.3	130.6	68.6	87.35
Non-fibrous carbohydrates (NFC)	101.8	95.4	363.0	432.9	418.2	453.8
Total phenols (mg kg ⁻¹)	855.0	1710.0	-	223.7	230.8	253.5
Total flavonoids, (mg kg ⁻¹)	593.51	1621.35	-	-	-	-
Total tannins, (g kg ⁻¹)	6.3	12.6	-	1.4	2.8	3.5

¹Calcium (Max.) 220.00 g kg⁻¹; Calcium (Min.) 160.00 g kg⁻¹; Phosphorus (Min.) 40.00 g kg⁻¹; Magnesium (Min.) 6.00 g kg⁻¹; Sodium (Min.) 85.00 g kg⁻¹; Sulfur (Min.) 12.00 g kg⁻¹; Cobalt (Min.) 20.00 mg kg⁻¹; Copper (Min.) 520.00 mg kg⁻¹; Iodine (Min.) 25.00 mg kg⁻¹; Manganese (Min.) 650.00 mg kg⁻¹; Selenium (Min.) 10.00 mg kg⁻¹; Zinc (Min.) 2000.00 mg kg⁻¹; Fluoride (Max.) 400.00 mg kg⁻¹.

2.4. Sample Collection and Data: Slaughter Procedure, Data Collection and Meat Sampling

After 121 days of experimentation, 24 steers with an average body weight of 414 kg and 13 months of age were transported, 17 hours before slaughter, to a commercial slaughterhouse in the city of Chapecó, SC, Brazil, 25 km from the confinement area. Transport lasted about 1 hour. At the slaughterhouse, the animals remained in the covered waiting room for around 16 hours, with feed restriction and access to drinking water.

After rest period and water diet, the animals were directed to the containment box and stunned with a captive-bolt pistol. The slaughters followed current legislation and underwent veterinary inspection. The hot carcasses were weighed on a digital scale immediately after each carcass was dressed and washed. After 30 minutes of slaughter, pH was measured using a portable digital meter (TESTO[®], 205PH), between the 12th and 13th ribs in the left *Longissimus thoracis et lumborum* (LTL) muscle region. For calibration/adjustment of the measurement system (potentiometer), pH 7.00 and 4.01 commercial buffer solutions were used. A built-in temperature sensor ensured temperature compensation for the pH measurements. After slaughter, the carcasses were refrigerated and kept at 6.0 ± 0.2°C for 24 hours. After this period, the pH was measured again, and the LTL muscle on the left side was removed and transported by 30 min in a thermal box to the laboratory. Then, we began analyzing the meat.

Six 2-cm thick steaks were cut from the LTL from each sample aseptically with sterile knives in a controlled environment using a Bunsen burner. Of these, one was used immediately to determine the parameters of meat color, water-holding capacity, cooking loss, shear force, and FA profile; and another steak was used to analyze shelf life, representing the beginning of the period (day 1 - moment that corresponds to 24 hours after the slaughter of the animal). The remaining steaks were stored refrigerated (as described below) for analysis at different shelf-life periods. All meat analyzes were carried out at the Animal Nutrition Laboratory and Microbiology Laboratory of the Universidade do Estado de Santa Catarina, UDESC, Chapecó, Brazil.

2.5. Laboratory Analysis

2.5.1. Color, Water-Holding Capacity, Cooking Loss, and Shear Force

The steaks were cooked and after 2 minutes of exposure to ambient air, the surface color of the meat was measured using a portable gauging colorimeter (Konica Minolta®, CR-400) with a D65 illuminant and spectral reflectance included as calibration mode with an 8-mm observation aperture and an 11-mm illuminant/observer. Luminosity (L^*), redness (a^*), and yellowing (b^*) were measured at three randomly selected sample points. The values of the parameters were expressed as the mean of three measurements.

The water-holding capacity was measured using an adaptation of the methodology of [19]. 0.3 g of meat ground (by scraping the knife over the meat) was weighed and placed them on filter paper (15 × 15 cm) between two acrylic plates. A weight of 10 kg was placed on top of the sample for 5 minutes. After pressing, the meat was weighed, and water loss was calculated based on the weight difference. The result was expressed as a percentage.

Cooking loss and shear force analyses were performed using 2-cm steaks. Before cooking the meat, the sample was weighed and covered in aluminum foil. Cooking was carried out on a portable grill (Mondial® Due Grill Smart), preheated to 170°C until reaching an internal temperature of 75°C (measured using a culinary thermometer inserted in the geometric center of the sample during cooking). After cooking, the samples were removed from the aluminum foil and the final weight was obtained after temperature stabilization. according to the methodology adapted from researchers [19]. The percentage of cooking water loss (CL) was calculated as follows: [(weight of raw steak – weight of cooked steak)/weight of raw steak] × 100.

The cooked and chilled samples were cut into subsamples in the fiber's longitudinal direction. Using a digital caliper, the height and width of the subsamples were measured for later calculation of the area. The shear force was measured on each cuboid perpendicular to the fiber, using a texture analyzer (Stable Micro Systems®, TA-XT plus) coupled to a 1 mm thick Warner-Bratzler V-shaped cutting blade. The test speed was 3.30 mm s⁻¹ to measure the force required to cross-cut each cuboid, and the values were expressed as N per mm² of meat. All determinations were performed on two replicates per sample.

2.5.2. Shelf Life

The parameters to measure meat quality—microbiological analysis, color and oxidative state was evaluated at 1, 3, 7, and 10 days of shelf life (a proxy for retail exposure), with day 1 considered 24 hours after slaughter (chilled meat). The fatty acid profile was evaluated in meat only on days 1 and 10 of shelf life.

Four steaks (2 cm thick per experimental unit) were placed in polystyrene trays coated with a low-density polyethylene film (Figure 1). The trays were packed, labeled, and randomly placed in a controlled greenhouse, illuminated with white fluorescent light (Blumenau®, five LED Tube lamps, 9W, 6500 K, 900 lumens) with forced ventilation and a temperature of 4 ± 0.4°C. Lighting re-

mained on 12 hours a day, simulating retail display conditions. The wrapped trays were changed places daily to minimize differences in the incidence and intensity of light and possible changes in temperature inside the equipment.

2.5.3. Microbiology

Before the other analyses were conducted, a subsample of 10 g of meat was aseptically separated and weighed in sterile plastic bags for food and homogenized with 90 ml of buffered peptone water for 10 min on a horizontal orbital shaker table (LOGEN SCIENTIFIC LS2312). From this dilution (10^{-1}), 1 mL was transferred to tubes containing 9 mL of buffered peptone water, obtaining the other serial dilutions of 10^{-2} and 10^{-3} , used on days 1, 3, and 7 of analysis. For day 10, dilutions of 10^{-4} , 10^{-5} , and 10^{-6} were used. Total bacterial counts (total mesophils) were performed on standard count agar (SCA), and lactic acid bacteria counts were performed on de Man, Rogosa, and Sharpe agar (MRS) using the pour plate method. The three serial dilutions performed previously were used, where 1 mL of each dilution was inoculated into sterile Petri dishes. Subsequently, the SCA plates were incubated in a bacteriological oven at $37 \pm 1^\circ\text{C}$ for 24 h, and the MRS plates were placed in an anaerobic chamber (Panasonic[®], MCO-19AIC UV) in a 5% CO_2 atmosphere at $30 \pm 1^\circ\text{C}$ for 72 h. To count *Escherichia coli*/coliforms and *Enterobacteriaceae*, 3M Petrifilm[™] EC 6414 and EB 6420 plates were used, respectively. Initially, the 10^{-1} dilution was used, and later (day 10), the 10^{-3} dilution was used. The plates were incubated at $37 \pm 1^\circ\text{C}$ for 24 h. Bacterial counts were expressed as logarithms of colony-forming units per gram of meat ($\log \text{CFU g}^{-1}$ meat).



Figure 1. Illustration of steaks placed in polystyrene trays coated with low-density polyethylene film.

2.5.4. Oxidant/Antioxidant Status

From the central area of the steak, 0.5 grams were removed and homogenized in saline solution to determine the antioxidant/oxidant status. The samples were centrifuged for 10 min at 7500 g. The supernatants were collected and stored in microtubes at -20°C until analysis.

Meat glutathione S-transferase (GST) was measured based on Habig *et al.* [20] and was expressed as $\mu\text{mol CDNB min}^{-1} \text{mg}^{-1}$ of meat protein. Serum lipid peroxidation was measured based on the amount of thiobarbituric acid reactive substances (TBARS) according to literature [21], and the results were expressed as nmol MDA mL^{-1} . Determination of reactive oxygen species (ROS) in the meat homogenate was based on Halliwell and Gutteridge [22] and expressed as U DCFH mg^{-1} meat protein. According to Ellman [23], protein thiols were measured, and the results were expressed as $\text{nmol thiols mg}^{-1}$ of protein.

2.5.5. Fatty Acid (FA) Profile

The method of Bligh and Dyer [24] was modified for extraction of lipids from beef and animal feed samples. 1.5 g was added of bovine muscle samples, 0.5 mL of water, 5 mL of methanol, and 2.5 mL of chloroform into 15-mL polypropylene tubes, and mechanical shaking was performed for 60 min. Then, 2.5 mL of chloroform and Na_2SO_4 1.5% solution were added to promote a biphasic system. This mixture was shaken for 2 minutes and then centrifuged for 15 minutes at 2000 rpm. Lipids obtained from the chloroform phase were subjected to FA analysis.

FA methylation was performed by a transesterification method proposed by researchers [25]. We added 1 mL of 0.4 M KOH methanolic solution to the extracted lipids in a test tube and shook the mixture in a vortex for 1 min. Samples were kept in a water bath for 10 min at boiling point. Subsequently, the material was cooled at room temperature, and 3 mL of 1 M H_2SO_4 was added, shaken in a vortex, and maintained in a water bath for 10 min. After cooling, we added 2 mL of hexane and centrifuged at 2000 rpm for 10 min. Finally, hexane with the FA methyl esters (FAMES) was subjected to chromatography analysis.

For the FAME determination, a gas chromatograph model TRACE 1310 was equipped with a flame ionization detector (Thermo Scientific). One microliter of samples was injected in a splitsplitless injector operated in 1:20 ratio split mode at 250°C . Hydrogen was used as carrier gas at a constant flow rate of 1.5 mL min^{-1} . FAME separation was carried out using an RT 2560 chromatography column ($100 \text{ m} \times 0.25 \text{ mm} \times 0.20 \mu\text{m}$ thickness film, Restek, USA). The FAME compounds were identified by comparing experimental retention times with those of an authentic standard (FAME Mix-37, Sigma Aldrich, St. Louis, MO). The results were expressed as a percentage of each FA identified in the lipid fraction (Material Supplementary 1), considering the chain size equivalent factor of FAME for FID and conversion factor of ester to the respective acid, according to literature [26].

2.6. Statistical Analyses

All data were analyzed using the 'MIXED procedure' of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4), with the Satterthwaite approximation to determine

the denominator degrees of freedom for the test of fixed effects. The carcass weight, yield, meat pH, shear force, water-holding capacity, CL, and intestinal and liver antioxidant variables were tested for fixed effects of treatment using the animal (treatment) as the random effect. All other variables were analyzed as repeated measures and were tested for fixed effects of treatment, day, and treatment \times day, using the animal (treatment) as the random effect. The compound symmetry covariance structure was selected according to the lowest Akaike information criterion. Means were separated using the PDIFF method, and all results were reported as LSMEANS and standard errors. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10 .

3. Results

3.1. Carcass Characteristics

The GPS-fed steers had greater chilled carcass pH than the control animals ($P < 0.05$, **Table 2**); the animals that received the GPB diet had a pH similar to the steers in the control group. There were no diet differences for other carcass variables (weight, yield, and hot carcass pH) ($P > 0.05$).

3.2. Physicochemical Parameters of Meat

The experimental diets influenced the meat's chemical composition ($P < 0.05$). While there were no diet differences for DM content ($P > 0.05$), LTL from GPB fed steers has a greater crude protein content than LTL from steers fed the control diet ($P < 0.05$) (**Table 3**).

There were no diet differences for meat luminosity (L^*), redness (a^*), and yellowing (b^*) over various periods of simulated retail exposure ($P > 0.05$, **Table 3**). There was also no day effect for luminosity ($P = 0.63$) or red color ($P = 0.13$); however, there was a day effect for meat yellowness ($P < 0.001$) with lower values on day 1 than the other days (3, 7, and 10) for the 3 dietary treatments. There were no diet differences for Warner-Bratzler shear force, CL, and water-holding capacity ($P > 0.05$).

Table 2. Effects of diets containing grape pomace silage and grape pomace bran on carcass traits for Charolais \times Nellore steers

Items	Dietary Treatment (Treat) ¹			SEM	P-value	
	Control	GPS	GPB		Treat	Treat \times Day
Cold carcass weight (kg)	214	218	198	10.2	0.4	-
Dressing (%)	51.7	52.1	50.9	0.67	0.48	-
pH, hot carcass	6.5	6.52	6.64	0.05	0.22	-
pH, chilled carcass	5.30 ^b	5.48 ^a	5.35 ^{ab}	0.04	0.05	-

¹Dietary treatment: control diet (traditional confinement diet), GPS diet (inclusion of 100 g kg⁻¹ of grape pomace silage in the diet, DM basis), and GPB diet (inclusion of 100 g kg⁻¹ of grape pomace bran in the diet, DM basis). ^{a-b} Within a row, means differ ($P \leq 0.05$) or tend to differ ($0.05 < P \leq 0.10$).

Table 3. Physicochemical meat quality for beef from Charolais x Nellore steers.

Items	Dietary Treatment (Treat) ¹			SEM	P-value	
	Control	GPS	GPB		Treat	Treat × Day
Dry matter (g kg ⁻¹)	267	283	292	17.0	0.15	-
Crude protein (g kg ⁻¹)	234 ^b	249 ^{ab}	265 ^a	10.0	0.05	-
Warner-Bratzler shear force (WBSF) (kgf cm ²)	76.10	88.26	78.06	0.91	0.57	-
Water-holding capacity (WHC) (g water g ⁻¹ dry matter)	77.0	75.9	75.8	1.60	0.87	-
Cooking loss (CL) (g kg ⁻¹)	249	264	258	20.6	0.87	-
Lightness, (L*)					0.35	0.39
d 1	38.1	41.0	40.8	1.49		
d 3	40.9	42.8	42.3	1.49		
d 7	39.6	43.0	43.2	1.49		
d 10	38.2	41.9	39.4	1.49		
Average	39.2	42.2	41.4	1.37		
Redness, (a*)					0.40	0.54
d 1	12.4	13.9	13.6	0.71		
d 3	13.4	15.1	13.7	0.71		
d 7	11.9	13.2	12.0	0.71		
d 10	11.5	11.4	12.0	0.71		
Average	12.3	13.4	12.8	0.54		
Yellowness, (b*)					0.27	0.88
d 1	6.53	7.85	7.57	0.78		
d 3	11.8	12.4	12.2	0.78		
d 7	10.4	12.3	12.6	0.78		
d 10	10.5	12.1	11.8	0.78		
Average	9.79	11.2	11.04	0.60		

¹Dietary treatment: control diet (traditional confinement diet), GPS diet (inclusion of 100 g kg⁻¹ of grape pomace silage in the diet, DM basis), and GPB diet (inclusion of 100 g kg⁻¹ of grape pomace bran in the diet, DM basis). ^{a-b} Within a row, means differ (P ≤ 0.05) or tend to differ (0.05 < P ≤ 0.10).

3.3. Oxidative Status of Meat

There were diet effects for oxidative and antioxidant system indicators (**Table 4**). The TBARS and ROS were lower for GPS and GPB treatments than the control (P < 0.05). There was a treatment x day interaction (P = 0.04) for GST activity in meat; LTL from cattle fed GPS and GPB had greater GST activities on day 1 than LTL from cattle fed the control diet. The GST activity tended (P = 0.07) to be greater in LTL from cattle fed GPS and GPB versus LTL from cattle fed the

control diet. There was a day effect ($P < 0.01$) for TBARS, ROS, and GST for all diets, with a linear and increasing trend over days stored for levels of TBARS and ROS. There was a linear and decreasing trend over days stored for GST activity. There were no treatments, treatment \times day, or day effects ($P > 0.05$) for protein thiols.

Table 4. Effects of feeding grape pomace silage or grape pomace bran on oxidative status of beef maintained under retail exposure conditions.

Items	Dietary Treatment (Treat) ¹			SEM	P-value	
	Control	GPS	GPB		Control	Treat \times Day
TBARS ²					0.03	0.01
d 1	8.74	8.17	8.29	0.32		
d 3	9.23 ^a	8.61 ^{ab}	8.31 ^b	0.32		
d 7	12.8 ^a	10.5 ^b	8.95 ^b	0.33		
d 10	14.8 ^a	11.1 ^b	11.3 ^b	0.32		
Average	11.4 ^a	9.59 ^b	9.21 ^b	0.27		
GST ³					0.07	0.04
d 1	521 ^b	594 ^a	586 ^a	21.0		
d 3	496 ^b	587 ^a	556 ^{ab}	36.0		
d 7	457	501	521	38.0		
d 10	369	374	354	37.0		
Average	465 ^b	514 ^a	504 ^a	35.3		
PSH ⁴					0.93	0.96
d 1	5.36	6.29	5.95	0.60		
d 3	5.40	5.40	5.66	0.60		
d 7	4.82	4.79	4.78	0.60		
d 10	5.63	5.29	5.57	0.60		
Average	5.30	5.45	5.49	0.37		
ROS ⁵					0.001	0.002
d 1	685 ^a	502 ^b	512 ^b	61.2		
d 3	731 ^a	589 ^b	573 ^b	61.3		
d 7	896 ^a	632 ^b	590 ^b	63.0		
d 10	1056 ^a	877 ^b	878 ^b	64.3		
Average	842 ^a	650 ^b	638 ^b	56.4		

¹Dietary treatment: control diet (traditional confinement diet), GPS diet (inclusion of 100 g kg⁻¹ of grape pomace silage in the diet, DM basis), and GPB diet (inclusion of 100 g kg⁻¹ of grape pomace bran in the diet, DM basis). ²Thiobarbituric acid reactive substances (nmol MDA/mL); ³Glutathione S-Transferase (μ mol CDNB/min/mg protein); ⁴Protein thiols (μ mol SH/mg of protein); ⁵Reactive oxygen species (U DCFH/mg protein); ^{a-b} Within a row, means differ ($P \leq 0.05$) or tend to differ ($0.05 < P \leq 0.10$).

3.4. Microbiology

The bacterial counts are shown in **Table 5**. Diets did not influence total mesophiles or lactic acid bacteria counts at various simulated retail exposure periods ($P > 0.05$). There were diet effects ($P < 0.05$), but no treatment \times day interactions ($P > 0.05$) for total coliforms (TC) and enterobacteria (ENTB). The LTL from steers fed GPS had lower TC counts than LTL from steers fed the control diet. The TC counts for LTL from steers fed GPB were similar to counts from LTL from steers fed GPS or control diets. There was a day effect ($P < 0.001$) for TC, ENTB, and total mesophile counts in LTL from cattle fed all diets, with an increasing trend over the days of meat exposed to simulated retail conditions. Likewise, there was a day effect ($P < 0.001$) for lactic acid concentrations, which increased linearly over time.

3.5. FA Profile

Small modulations were observed by the inclusion of different experimental diets on the lipid profile in fresh meat at 24 h post-slaughter (**Table 6**). The GPS and GPB diets increased cholesterol-lowering fatty acids CLFA (cis-oleic, linoleic and α -linolenic fatty acids) concentrations when the control diet ($P = 0.05$). The LTL from steers fed GPS had lower concentrations of SFA when compared to LTL for steers fed control and GPB diets ($P < 0.05$). There was a trend for greater amounts of C20:4 n6 in LTL from steers fed GPS and GPB diets than in steers fed the control diet ($P = 0.07$; **Table 6**). Steers fed BPG tended to have greater amounts of total omega 6 FA when compared to steers fed the control diet ($P = 0.08$). Steers fed GPS and GPB diets tended to have greater concentrations of C18:1 n9c than steers fed the control diet ($P = 0.10$). Total omega 9 FA concentrations were greater in LTL from steers fed GPS than steers fed GPB ($P = 0.10$). There were no diet effects on the amount of total monounsaturated fatty acids (MUFA) and PUFA and for the atherogenic index ($P > 0.05$).

Table 5. Microbiology for LTL from Charolais \times Nellore fed grape pomace silage and white grape pomace.

Items ²	Dietary Treatment (Treat) ¹			SEM	P-value	
	Control	GPS	GPB		Treat	Treat \times Day
Total coliforms					0.04	0.39
d 1	2.75	1.96	2.43	0.20		
d 3	2.73	2.14	1.88	0.20		
d 7	3.87	3.62	3.79	0.20		
d 10	5.42	4.65	5.29	0.20		
Average	3.69 ^a	3.17 ^b	3.35 ^{ab}	0.13		
Enterobacterium					0.10	0.51
d 1	2.36	2.08	2.21	0.19		
d 3	2.65	1.91	2.32	0.19		

Continued

d 7	3.72	3.80	3.85	0.19		
d 10	5.52	5.16	5.52	0.19		
Average	3.56 ^a	3.24 ^b	3.48 ^{ab}	0.12		
Total mesophiles					0.39	0.58
d 1	4.25	3.87	3.80	0.33		
d 3	4.04	3.77	3.85	0.33		
d 7	5.73	5.28	5.44	0.33		
d 10	6.88	6.31	7.48	0.33		
Average	5.22	4.81	5.14	0.23		
Lactic acid					0.56	0.83
d 1	3.67	3.26	3.27	0.29		
d 3	3.66	3.32	3.49	0.29		
d 7	5.60	5.20	5.06	0.29		
d 10	6.73	6.62	7.08	0.29		
Average	4.91	4.60	4.72	0.23		

¹Dietary treatment: control diet (traditional confinement diet), GPS diet (inclusion of 100 g kg⁻¹ of grape pomace silage in the diet, DM basis), and GPB diet (inclusion of 100 g kg⁻¹ of grape pomace bran in the diet, DM basis). ²Values expressed as log CFU/g meat. ^{a-b} Within a row, means differ ($P \leq 0.05$) or tend to differ ($0.05 < P \leq 0.10$).

Table 6. Fatty acid profile for LTL from Charolais x Nellore fed grape pomace silage and white grape pomace.

Items	Dietary Treatment (Treat) ¹			SEM	<i>P</i> -value	
	Control	GPS	GPB		Treat	Treat × Day
Intramuscular fat (mg/g muscle)	25.5	25.2	31.0	4.50	0.64	0.60
Σ_MCFA ²	39.2	37.9	37.4	0.75	0.25	0.79
Σ_LCFA ³	59.9 ^b	62.0 ^a	62.6 ^a	0.80	0.05	0.76
<i>Sum fatty acids</i>						
Total SFA	50.9 ^b	49.5 ^a	50.8 ^b	0.40	0.05	0.23
Total MUFA	41.5	42.5	40.2	0.95	0.29	0.87
Total PUFA	6.80	7.81	8.95	0.99	0.37	0.38
<i>Ω</i>3						
C22:6n3	0.11	0.09	0.10	0.02	0.76	0.70
Σ_n3_PUFA	0.79	0.71	0.74	0.10	0.87	0.55
<i>Ω</i>6						
C18:2n6c	4.47	5.15	5.89	0.55	0.28	0.53
C18:3n6	0.02	0.03	0.03	0.007	0.62	0.98
C20:3n6	0.29	0.34	0.42	0.06	0.39	0.52

Continued

C20:4n6					0.07	0.08
d 1	0.89 ^b	1.66 ^a	1.57 ^a	0.25		
d 10	1.13 ^b	1.30 ^b	1.98 ^a	0.25		
Average	1.01 ^c	1.48 ^b	1.78 ^a	0.19		
Σ _n6_PUFA	5.93 ^b	7.00 ^{ab}	8.12 ^a	0.68	0.08	0.17
<i>Omega 9</i>						
C18:1n9c	35.5 ^b	37.2 ^a	35.4 ^b	0.64	0.10	0.83
C20:1n9	0.13	0.12	0.17	0.03	0.63	0.44
C24:1n9	0.01	0.009	0.01	0.003	0.68	0.81
Σ _n9_PUFA	37.1 ^{ab}	38.6 ^a	36.7 ^b	0.73	0.10	0.77
<i>Health Indexes</i>						
Atherogenic index	10.9	10.4	9.99	0.92	0.78	0.27
Δ 9_Desaturase_C18					0.08	0.05
d 1	70.1 ^b	73.3 ^a	69.4 ^b	0.88		
d 10	71.0	71.1	69.0	0.88		
Average	70.5 ^{ab}	72.2 ^a	69.2 ^b	0.84		

¹Dietary treatment: control diet (traditional confinement diet), GPS diet (inclusion of 100 g kg⁻¹ of grape pomace silage in the diet, DM basis), and GPB diet (inclusion of 100 g kg⁻¹ of grape pomace bran in the diet, DM basis). ²MCFA: *midium-chain fatty acids*; ³LCFA: *long-chain fatty acids*. ^{a-b} Within a row, means differ ($P \leq 0.05$) or tend to differ ($0.05 < P \leq 0.10$).

4. Discussion

The present study is the first to conduct a joint evaluation comparing the inclusion of dehydrated and ensiled grape residue in the diet of Charolais x Nelore steers, focusing on the FA profile, antioxidant activity, microbiology, and chemical-physical profile of fresh beef exposed to simulated shelf life. The animal growth rate, through its effect on fat formation, can strongly influence the FA composition [27]. In this study, growth rate was lower than GPB diet compared to control and GPS diets [18] despite all diets being isonitrogenous and isoenergetic composition (Table 1).

The absence of a diet effect for meat color attributes (a^* L^* and b^*) is similar to previous findings in the examining the inclusion of pomace residue from grapes in sheep [28] [29], pomace residue from grape in cattle [30] and grape seed in sheep [31]. In the present study, it was not possible to observe a day effect for the variables L^* and a^* that represent inhibition of protein denaturation and conversion of deoxymyoglobin to oxymyoglobin and their effects on the increased indicators of luminosity [32] and red coloration [33]. The addition of ensiled or dehydrated grape residue in the diet of cattle, although showing good antioxidant capacity due to low levels of TBARS and ROS and high activity of the GST enzyme, did not have the potential to preserve red color until the end of

the shelf life, which presumes oxidation of myoglobin or oxymyoglobin to metmyoglobin [33]. This effect was minimized with higher vitamin E concentrations in lamb [34]. It is worth mentioning that, even with no difference in color attributes, the luminosity and redness values observed in the present study were in the ideal range (35.3 to 46.3 and <14.5 for L* and a*, respectively) considered acceptable by beef consumers [35] [36].

The inclusion of grape residue in the steers' diets influenced the FA profile in the meat. We associated the highest amounts of LCFA in the meat of steers that received the GPS and GPB diets due to the increased proportion of the long-chain FA C18:2n6c (Supplementary material 1). In addition to the differentiated lipid profile of the residue, which directly affects the FA profile of the experimental feed (and, therefore, what the animal consumes), the grape residue has large amounts of polyphenols, which influence biohydrogenation and PUFA content in meat [16] [37]. The higher proportion of some n-6 PUFAs was observed in GPS and GPB diets; what can be attributed to higher levels of polyphenol compounds in the diets that reduce ruminal lipolysis and protect PUFAs from biohydrogenation in the rumen, making them inaccessible to microbes or their enzymes [16] [38]. The investigation for increased MUFA and PUFA content and concomitant declines in SFA content in animal products have been the subject of several studies, given the relationship between dietary FA and human health [39]. Diets rich in saturated FA (SFA) contribute to an increase in the level of LDL-cholesterol, which is positively correlated with diseases of the circulatory system [40], in addition to obesity [41]. In contrast, some MUFAs and PUFAs (particularly long-chain n-3 PUFAs) improve human health [42] [43]. Thus, although the differences in total PUFA concentrations are not significant (except some for n-6 and 9), linked to lower SFA concentrations in the GPS group, there is a positive effect of including the residue in the ensiled and dehydrated form, even with changes and interferences of ruminal biohydrogenation [44]. This phenomenon results in higher levels of SFA, compared (for example) with swine meat [45]. Steers that consumed BPG had a greater concentration of protein in beef LTL, an intriguing result that needs to be further studied to understand the mechanisms involved that contributed to this finding regarding alterations to beef chemical composition.

Lipid stability is a dominant determinant of meat quality during shelf life [46] because lipid peroxidation is a primary deterioration mechanism, especially for meat products [47]. In the present study, lipid peroxidation and ROS concentrations were lower in beef from steers fed the GPS and GPB diets. These findings agree with [30], who added 150 g kg⁻¹ of dry grape pomace to the diet of Angus cattle and observed lower lipid oxidation and higher antioxidant activity in meat. Similar effects were observed in lamb [28] [34] [48] and chicken [49]. These findings corroborate the literature that describes the antioxidant activities and capacity of grape phenolics [50] [51]. We believe that the effects observed in the meat of these animals are due to the vigorous H⁺ donor activity [52], the ability to scavenge free radicals and chelate pro-oxidants (transition metals), and singlet

oxygen quenching [53] [54].

The antimicrobial and antioxidant potential of grape residue in the meat of animals depends solely on the ingestion of these compounds and their bioavailability, such that there is the accumulation of these compounds or their metabolites in muscle tissues during an animal's life. For polymeric and high molecular weight substances such as condensed tannins, absorption may be limited, and it is unlikely that oligomers larger than trimers can be absorbed in the small intestine in their native form [55]. Therefore, we believe monomers such as catechins, quercetin, epicatechin, and epicatechin-3-O-gallate provide the antimicrobial and antioxidant effects [56]-[59]. Nevertheless, we cannot rule out the possible biodegradation of polymeric proanthocyanidins, in which they are metabolized into bioavailable compounds with an intact flavonoid ring structure (epicatechin) [48].

Phenolic compounds (primarily hydroxycinnamic acids, gallic acid, flavonols, flavan-3-ols, and trans-resveratrol) make up the grape and its by-products and provide antimicrobial activity [60]. This inhibitory potential for microbial proliferation occurs by accumulating these compounds in the lipid bilayer, causing alteration and disarrangement in the membrane, altering and compromising its function. The increase in membrane permeability allows entry into the bacterial cell, exerting inhibitory activity in the cell cytoplasm, leading to lysis and release of intracellular ATP [61] [62]. The hydroxyl groups of phenols are primarily responsible for inhibitory activity and can lyse the bacterial cell membrane [63] [64]. Catechins are highly potent inhibitors of DNA gyrase, vital for DNA transcription and replication and chromosomal segregation of bacteria [65].

Therefore, we associated the lower count of TC and total mesophiles in beef from steers fed the GPS diet with the ability of phenolic compounds to inhibit bacterial proliferation. Our enterobacterial counts are similar to those reported by Viera *et al.* [66], who included 50 g kg⁻¹ DM from the total grape pomace diet in the lamb diets. In contrast, other researchers [28] [34] found no effect in lamb; however, these authors observed a lower total viable count in the meat. According to literature, was observed lower TC counts with the addition of grape residue [30], similar to the present study.

Although the GPS diet showed an antibacterial effect on the meat, this effect was insufficient to prolong the shelf life of the meat until the seventh day. According to Normative Instruction 60 (IN60) of microbiological food standards, followed in Brazil, meat from all experimental groups would be outside the acceptable standard on the seventh day of shelf life. On the seventh day, all groups reached a plateau of 5 log CFU g⁻¹ of meat for total mesophile counts, with the control group tending to have higher counts, followed by the GPB and GPS groups. Although we did not analyze it, we speculate that a possible effect for the GPS diet could be observed on the sixth day, given the bacterial growth behavior and antimicrobial effect on the GPS experimental group. The International Commission on Microbiological Specifications for Food (ICMSF) guidelines suggest a maximum bacterial count limit of 7 log CFU/g, in contrast to IN60

which suggests a maximum bacterial count of 5 log CFU/g. Thus, according to the ICMSF, microbiological counts did not exceed the maximum count limits until the tenth day in the control and GPS groups. Although we did not perform a sensory panel, we considered the meat unacceptable for consumption on the seventh day, given the strong odor. To support our finding, [67] conducted a sensory panel of meat from raw beef distributed in Korea, and found lower bacterial counts suggested as a critical limit by the ICMSF.

Complementary study published by Molosse *et al.* [18] presents evaluations of performance, animal health, and the digestibility of steers. The inclusion of GPS had a positive impact to animal, as it stimulates and modulates systems and antioxidants. Furthermore, it is important to make it clear that the consumption of grape residue was included in the diet as an ingredient, i.e., nutritional function, without affecting the daily feed intake of these cattle [18].

5. Conclusion

Dietary treatments with the inclusion of GPS and GPB decreased oxidation of beef lipids, as well as improved the nutritional value, due to the greater amount of FA beneficial to human health. In addition, feeding the GPS diet reduced bacterial counts in beef over the course of retail meat exposure. In this sense, we can partly validate our hypothesis; the feeding the grape by-product residues improved beef quality. However, feeding GPB did not further improve beef quality in comparison to feeding GPS. Therefore, we conclude that the inclusion of these residues replacing other traditional fiber sources in the diet of beef cattle in the finishing phase can serve as an antioxidant source in meat products, denoting a positive effect on meats that are exposed to retail.

Funding

This study was supported by the UDESC and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) of Santa Catarina state; and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

Data availability

The data analysed in this investigation are available upon request to the corresponding author.

Ethics Approval

The project was approved by the ethics committee on the use of animals in research of UDESC (CEUA n° 4948210322). The study followed the guidelines of the Brazilian Council for Animal Experimentation, Brazil.

Consent for Publication

All authors have consented to the publication and presentation of the data in this article.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplement

Supplementary material 1. Feed ingredient and dietary treatment fatty acid composition (% of total fatty acids)

Item ¹	Ingredient			Dietary Treatment ²			
	GPS	GPB	WB	Control	GPS	GPB	<i>P-diet</i>
C12:0	0.10	0.10	0.10	0.15	0.17	0.13	0.68
C14:0	0.3	0.20	0.10	0.23	0.24	0.27	0.75
C15:0	-	-	0.10	0.07	0.05	0.06	0.92
C16:0	12.7	10.9	19.3	23.99 ^a	19.08 ^b	20.60 ^{ab}	0.04
C16:1	0.4	0.6	0.2	0.13 ^b	0.19 ^{ab}	0.26 ^a	0.01
C17:0	0.1	0.1	0.2	0.23	0.17	0.17	0.12
C18:0	5.8	5.7	2.9	6.82	6.36	7.01	0.53
C18:1n9c	21.6	17.9	21.6	25.93	24.16	24.15	0.91
C18:2n6c	56.2	61.6	50.6	36.22 ^b	43.65 ^a	42.35 ^a	0.02
C20:0	-	-	-	0.78	0.99	0.81	0.15
C20:1n9	-	0.1	0.7	0.37	0.27	0.26	0.36
C18:3n3	1.6	1.0	3.2	2.68	2.65	1.83	0.10
C21:0	-	-	-	0.31 ^a	0.11 ^b	0.25 ^a	0.01
C20:2	-	-	0.1	0.30 ^a	0.15 ^b	0.24 ^a	0.01
C22:0	0.7	0.8	0.3	0.58 ^b	0.71 ^a	0.56 ^b	0.03
C20:3n3	0.1	0.1	0.2	0.17	0.15	0.12	0.90
C20:4n6	-	-	-	0.10	0.05	0.05	0.87
C24:0	0.4	0.7	0.3	0.70	0.73	0.62	0.39
C24:1n9	-	-	0.1	0.23	0.13	0.25	0.14

¹C12:0 (Lauric), C14:0 (Myristic), C15:0 (Pentadecanoic), C16:0 (Palmitic), C16:1 (Palmitoleic), C17:0 (Heptadecanoic), C18:0 (Stearic), C18:1n9c (Oleic), C18:2n6c (Linoleic), C20:0 (Arachidic), C20:1n9 (cis-11-Eicosenoic), C18:3n3 (α-Linolenic), C21:0 (Henicosanoic), C20:2 (cis-11,14-Eicosadienoic), C22:0 (Behenic), C20:3n3 (cis-11,14,17-Eicosatrienoic), C20:4n6 (Arachidonic), C24:0 (Lignoceric), C24:1n9 (Nervonic). ²Dietary treatment: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg of grape pomace silage in the diet, DM basis), and GPB diet (inclusion of 100 g/kg of grape pomace bran in the diet, DM basis).