



Polyunsaturated fatty acid, volatile and sensory profiles of beef from steers fed citrus pulp or grape pomace

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ABSTRACT

The present study compared the effects of feeding dried grape pomace (DGP) or citrus pulp (DCP) at 150 g/kg dry matter compared to a control diet on major polyunsaturated fatty acids (PUFA), volatile and sensory profiles of beef. Feeding DGP or DCP diets to Angus steers for 90 d increased the proportions of C18:2n-6, C20:4n-6, C18:3n-3, total conjugated linoleic acid (CLA), n-3 and n-6 PUFA in muscle. Control-fed beef had greater concentrations of C18:1n-9, total aldehydes, ketones, and alcohols compared to DCP and DGP. Feeding DGP and DCP diets produced less tender beef than control. Overall, finishing steers on diets containing DGP or DCP compared to control increased proportions of total CLA, n-3 and n-6 PUFA, and reduced concentrations of aldehydes, ketones, and alcohols, but did not affect beef sensory attributes except for a slight reduction in tenderness.

1. Introduction

Tenderness, juiciness and flavor are key determinants of meat palatability and acceptability by the consumers (Legako et al., 2015; Prieto, Dugan, Larsen, Vahmani, & Aalhus, 2017). Fresh raw meat has little odor and only a mild serum like taste, which is described as salty, metallic and “bloody like” with a sweet aroma (Karabagias, 2018). During thermal processing, meat flavor develops as a result of complex interactions between precursors (i.e., free amino acids, free sugars, fatty acids, nucleotides and thiamine) producing volatile compounds occurring mainly through oxidation, Maillard reaction, Strecker degradation and/or the Ehrlich pathway (Elmore et al., 2005; Mottram, 1998; Spinnler, 2011). The content and composition of fatty acids [i.e., linoleic acid, C18:2n-6, α -linolenic acid, C18:3n-3, oleic acid, C18:1n-9 and other polyunsaturated fatty acids (PUFA)] are the main factors which influence flavor development in meat (Mezgebo et al., 2017; Piao et al., 2019). Fatty acid and volatile profiles are, therefore, of great importance to the processing, storage, transportation and marketing of fresh raw meat, and ultimately consumer sensory quality (Mottram, 1998; O’Quinn et al., 2016; Xu et al., 2019).

Dietary polyphenols can modulate ruminal fatty acid profiles

(Buccioni et al., 2017; Vasta et al., 2019), and contribute to the formation of volatile compounds in beef (Ianni et al., 2019). Polyphenols have been reported to protect dietary PUFA from biohydrogenation in the rumen, and/or inhibit growth and metabolism of ruminal bacteria responsible for biohydrogenation, thereby, enhancing tissue deposition of PUFA and their biohydrogenation intermediates (Vahmani et al., 2020; Vasta et al., 2019). Dietary polyphenols also improve protein digestibility and growth of ruminants by protecting proteins in the diet from degradation in the rumen and inhibiting the growth and metabolism of proteolytic bacteria (Mueller-Harvey et al., 2019; Tayengwa, Chikwanha, Dugan et al., 2020a). Decreased degradation of dietary protein in the rumen enhances its availability in abomasum and increases amino acid absorption in the ileum, and consequently its tissue deposition (Mueller-Harvey et al., 2019; Tayengwa, Chikwanha, Dugan et al., 2020a). Owing to their antioxidant and antimicrobial properties, feeding polyphenolic-rich diets [e.g., dried grape pomace (DGP) or citrus pulp (DCP)] have also been shown to extend the shelf life of lamb meat (Chikwanha, Muchenje, Nolte, Dugan, & Mapiye, 2019; Inserra et al., 2014) and beef (Ianni et al., 2019; Tayengwa, Chikwanha, Gouws, et al., 2020b). Scientific information linking beef consumers’ preferences with specific flavor attributes, originating from differences in

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feeding these fruit by-products is, however, lacking. Given DGP has more proanthocyanidins compared to DCP (Tayengwa, Chikwanha, Dugan et al., 2020a) and both these would be more than a control, it was hypothesized that feeding DGP and DCP would lead to increased proportions of PUFA and their biohydrogenation intermediates, and decreased volatile compounds associated with lipid oxidation in fresh beef without compromising sensory profile. Hence, the present study compared the effects of feeding Angus steers diets containing DGP or DCP to a control diet on PUFA composition, concentrations of volatile compounds and sensory profile of beef.

2. Materials and methods

2.1. Animals, diets and experimental design

The animal experiments were approved (ACU-2018-6738) by the Stellenbosch University Research Ethics Committee of the animal experimentation and were performed following the guidelines of the

South African National Standard. The animal management, diets and experimental design were reported by Tayengwa, Chikwanha, Dugan et al. (2020a) and Tayengwa, Chikwanha, Gouws, et al., (2020b). Briefly, twenty-four Angus steers with an initial body weight of 281 ± 15.4 kg and seven months of age were allotted to dietary treatments (8 steers/ treatment) in a completely randomized design for a feeding period of 90-d preceded by a 21-day adaptation period. The feed was provided as pelleted total mixed rations once daily at 0700. The amount of feed offered and refused per steer was measured daily to determine dry matter intake. The dietary samples were collected every day, pooled every seven days within animal and frozen at -20 °C. Post-trial, feed samples were dried, ground and stored at 4 °C until required for analyses. Steers were weighted biweekly.

2.2. Chemical, fiber, fatty acid, and phytochemical analyses for dietary ingredients and treatments

Preparation and analyses of chemical, fiber, phytochemical and fatty

Table 1
Proximate composition, and fatty acid profiles of dietary ingredients and treatments.

Item	DCP ¹	DGP ²	WB ³	Treatments			SEM ⁴
				Control diet	DCP diet	DGP diet	
Ingredients (g/kg)							
Dried citrus pulp (DCP)				–	150	–	
Dried grape pulp (DGP)				–	–	150	
White maize				313.6	335.3	365.1	
Canola oil cake				106.4	141.9	138.0	
Molasses				80.0	80.0	80.0	
Lucerne				100.0	100.0	100.0	
Wheat straw				90.0	90.0	90.0	
Wheat bran (WB)				231.7	50.0	51.4	
Gluten				50.0	42.0	–	
Limestone ⁵				19.7	2.2	16.9	
Chemical composition (g/kg)							
Dry matter (DM)	862.8	899.3	901.2	879.2	875.9	882.8	0.62
Ash	49.0	59.3	58.8	107.5	51.7	64.7	0.58
Crude protein	47.7	111.0	175.5	119.5	119.1	119.4	0.66
Starch	57.5	23.0	22.6	217.9	235.3	212.0	0.72
Ether extract	18.0	74.3	45.5	23.7	24.3	33.5	0.82
Ash free neutral detergent fiber (aNDFom)	145.8	317.6	385.4	164.4	172.7	183.5	1.41
Acid detergent lignin (ADL, [sa])	6.2	205.9	41.4	21.7	19.2	48.0	0.71
Metabolizable energy (MJ/ kg)	11.1	8.2	11.3	113.1	119.5	114.1	0.58
Non-fibrous carbohydrates (NFC)	739.5	437.8	342.7	584.5	632.2	598.9	0.58
Pectin + sugar	683.0	414.8	320.1	367.0	396.9	386.9	0.47
Mineral composition (g/kg)							
Calcium	17.3	3.1	1.5	11.9	7.4	10.7	2.4
Phosphorus	1.1	2.7	10.9	4.4	4.1	3.9	0.3
Potassium	8.3	18.6	12.4	11.3	11.5	12.3	0.6
Magnesium	1.1	1.1	4.4	2.9	2.6	2.7	0.1
Sodium	0.09	0.1	0.6	1.8	1.5	1.7	0.2
Iron	1.2	0.3	0.2	0.3	0.3	0.3	0.02
Copper, mg/kg	3.1	19.1	12.2	14.3	18.6	13.9	2.6
Zinc, mg/kg	5.8	12.2	86.7	105.9	78.9	87.9	13.8
Manganese, mg/kg	19.8	11.4	–	70.1	52.6	62.9	8.7
Total phenolics, g of gallic acid equivalent/kg DM	51.4	177.3	–	7.4	32.5	89.6	4.3
Total tannins, g of gallic acid equivalent/kg DM	19.1	104.2	–	–	12.7	50.7	0.47
Condensed tannins, % leucocyanidin equivalent	8.1	33.3	–	–	5.7	24.1	0.47
Ascorbic acid, mg/ kg	427.0	174.0	–	–	316.1	114.2	0.46
Alpha-tocopherol, mg/kg	74	47	–	9.8	16.8	12.6	0.12
Fatty acid composition (mg/g feed)							
C16:0	167.1	160.2	–	82.1	85.3	73.2	0.07
C18:0	23.1	10.3	–	4.4	6.3	6.3	0.04
C18:1n-9	83.3	46.4	–	120.1	136.3	111.4	0.47
C18:2n-6	562.2	589.1	–	666.4	635.3	693.3	0.17
C18:3n-3	82.1	76.3	–	92.2	97.2	64.3	0.07

Note: Table 1 was adapted from Tayengwa et al. (2020a, b).

¹ DCP means dried citrus pulp.

² DGP means dried grape pomace.

³ WB means wheat bran. (WB).

⁴ SEM: Standard error of mean.

⁵ The diet also contained urea (5 g/kg), salt (3.0 g/kg) and Fintech premix (0.6 g/kg).

acid composition of DCP, DGP and experimental diets presented in Table 1 were detailed by Tayengwa, Chikwanha, Dugan et al. (2020a) and Tayengwa, Chikwanha, Gouws, et al. (2020b).

2.3. Slaughter procedures and collection of meat samples

A day before slaughter, animals were transported 83 km to Worcester commercial abattoir (85°36'63"S, 98°19'46"E), South Africa. At the abattoir, the steers were deprived of feed for 16 h but water was always available. Animals were stunned with a non-penetrating captive-bolt, exsanguinated, and dressed in a commercial manner as regulated by the Meat Safety Act (2000) of South Africa. After slaughter, all the carcasses were weighed, halved, and chilled overnight at 4 °C. The left and right *longissimus thoracis et lumborum* (LTL) muscles were removed from the carcasses. For determination of fatty acids, 2.5 cm thick steaks from each steer were removed from the left posterior *longissimus thoracis* (LT) muscle, trimmed of visible fat and connective tissue, homogenized, and stored at -80 °C until required for analysis. The left posterior LT was also sampled for the determination of beef volatile compounds. A 2.0 ± 0.05 g sample of raw beef was placed into solid-phase micro-extraction (SPME) headspace vials sealed with polytetrafluoroethylene-faced silicone septa. The sealed vials were then frozen at -80 °C pending for further analysis. For sensory evaluation, all the right LTL were vacuum-packed in oxygen barrier bags having a gas permeability (at 23 °C and 75% RH) below 50 ml m² day⁻¹ atm⁻¹ and adequately sealed to prevent air entry, and frozen at -20 °C pending analysis.

2.4. Intramuscular fatty acid analysis

Intramuscular fatty acids of the LT muscles were extracted as described by Folch, Lees, and Sloane-Stanley (1957). Briefly, 1 g of LT muscle was homogenized with 20 ml chloroform-methanol (2:1; v/v) solvent that contained an antioxidant (0.01% butylated hydroxytoluene) and internal standard (0.5 ml of 10 mg C17:0/ mL solution). The homogenate was then filtered through Whatman's No. 1 glass microfibre filter paper into a 50 ml glass separatory flask. An aliquot of the lipid extract solution (250 µl) was allowed to evaporate to dryness under constant nitrogen flow in a water bath at 45 °C for 10 min and subsequently transmethylated at 70 °C for 2 h using 2 ml of a methanol:sulfuric acid (19.5:0.5, v/v) solution as the transmethylating agent. After cooling at room temperature, the FAMES were extracted with 1 ml water using 2 ml hexane and the hexane extract was allowed dry in a water bath at 45 °C under the constant flow of nitrogen. Thereafter, the dried FAME samples were resuspended in 100 µl hexane and transferred to gas chromatography (GC) vials for analysis.

The FAMES were analyzed using a Thermo Scientific TRACE™ 1300 gas chromatograph (GC, ThermoQuest, Milan, Italy) equipped with a flame-ionization detector (FID; ThermoQuest, Milan, Italy) coupled to a CTC analytics PAL autosampler. Separation of FAMES were accomplished on a TR-FAME capillary column (60 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent Technologies, CA, USA), with hydrogen as the carrier gas (Flow rate-1 ml/min). The GC conditions were as follows: the oven temperature was programmed at 50 °C and held for 2 min, increased by 25 °C/min to 180 °C, held for 5 min, and then increased up to 260 °C at 3 °C/min, held for 2 min. The temperature of injector was set at 240 °C with an injection volume of 1 µl using a 5:1 split ratio. The detector (FID) temperature was set at 250 °C. The individual FAME peaks were identified by comparing their retention times with mixtures of standard FAME (Supelco™ 37 Component FAME mix, Cat no. CRM47885, Supelco, USA). The FAMES were quantified using chromatographic peak areas and internal standard based calculations. The individual fatty acids concentrations were expressed as percentage of total fatty acids of fresh meat.

2.5. Volatile compound analysis of fresh raw meat

Solid-phase microextraction (SPME) and GC/MS were used for the analysis of the volatile profile of fresh raw beef samples (Tao, Wu, Zhou, Gu, & Wu, 2014). Briefly, glass vials containing samples were thawed overnight and 100 µl of 3-octanol and Anisole solution (1000 µg/kg) was added as internal standard. The glass vials were then placed in a 65 °C (±2 °C) water bath (Thermo Scientific, Waltham, MA, USA) and allowed to equilibrate for 5 min. Following equilibration, a SPME fiber was placed in vial headspace above the sample for 10 min. The fiber was coated with a 50/30 µm thick DVB/Carboxen/PDMS (Supelco, Bellefonte, PA; 57328-U) and pre-conditioned by heating it in a GC injection port at 270 °C for 30 min. After adsorption for 10 min, the fiber was removed from the vials and immediately inserted into the GC (TRACE 2000, Thermo-Finnigan, San Jose, CA). The GC injector and the desorption time were set at 250 °C and 10 min, respectively. The injector operated in splitless mode at 250 °C. The extracted volatile compounds were separated through a Supelco SPB 5 column (30 m × 0.25 mm i.d., film thickness 0.25 µm, Phenomenex, Cheshire, UK). The initial GC oven temperature was set of 40 °C held for 5 min, followed by an increase to 240 °C at 3 °C/min, and finally further increased to 250 °C at 5 °C/min (2 min hold) with a total acquisition program of 47 min. The transfer line and ion source temperatures were maintained at 250 °C. The helium was used as a carrier gas with a constant flow rate of 1.0 ml/min. The volatile compounds mass spectra (MS) were generated by using an Agilent MS detector equipped with an ion trap (Polaris Q, Thermo-Finishing, San Jose, CA); working with the electronic impact mode (70 eV) and full scan mode which was ranging from 33 to 500 m/z. The temperatures of MS quadrupole were maintained at 150 °C and that of MS source at 240 °C, respectively.

The Xcalibur™ Software from Thermo Fisher Scientific (Massachusetts, France) was used to process the chromatograms. The concentration of the volatile compounds were expressed as µg/kg meat and calculated by comparison of their peak areas with that of the internal standard, Anisole-d8 was used as the internal standard for the quantification of all chemical groups apart from the alcohols, for which 3-octanol was used. Volatile compounds were tentatively identified by comparing their retention indices (RIs) and mass spectra of the house database (Wiley 6/NIST 11, <https://webook.nist.gov>, Gaithersburg, USA) In addition, RI were compared to published literature indices data value on polar column (DB-WAX). Retention indices of the compounds were determined by running a series of n-alkanes (C5-C18) under the same chromatographic conditions and calculated according to Tao et al. (2014) using the following formula:

$$RI = [Rt(i) - Rt(n)/(Rt(n + 1) - Rt(n) + n)] \times 100$$

where: Rt(i) is the retention time (RT) of each targeted compound (i), Rt (n) and Rt(n + 1) are the RTs of the n-alkanes eluting directly before and after the targeted compound (i) under the same chromatographic conditions. The n is the carbon number of the n-alkane eluting before the targeted compound (i).

2.6. Descriptive sensory analysis

For sensory evaluation, the right LT was used for training the panel and LL for the test phase (i.e., data collection). The training phase was conducted over 4 days, with two sessions per day (8 sessions in total, each session lasted for 1 h) representing one replication/animal per treatment (3) per session, with the test phase following the same experimental design as for training. Prior to sensory evaluation, samples were thawed for overnight at 4 °C. All subcutaneous fat was removed, and each sample roasts was cut into approximately 35 cm to fit into individual coded oven roasting bags (GLAD® Medium 250 mm × 400 mm; Woolworths, Stellenbosch, South Africa). No seasonings were added to the roasts. A thermocouple probe, attached to a handheld

digital temperature monitor (Hanna Instruments, Bellville, South Africa), was inserted into the center of each meat roast (AMSA, 2015). The roasting bags were tied using twist ties and placed on a stainless-steel grid which was fitted on a stainless-steel oven-roasting pan. The roasts were roasted in an industrial oven (Hobart, Paris, France) preheated to 163 °C until an internal temperature of 70 °C was reached (AMSA, 2015). The roasts were immediately removed from the oven and cooled (room temperature was set at 21 °C) for 15 min before removal from the roasting bags. The roasts were then lightly blotted dry and cut into 1 cm³ cubes. To minimize variation among the cubes, the outer parts (browned meat surface) of the roasts were trimmed off and only the inner parts used for evaluation. Each cube was wrapped in aluminum foil and placed into a three-digit random coded glass ramekin. Each ramekin contained three wrapped meat cubes per treatment. Before serving the panelist, the meat was reheated inside the ramekins at 70 °C for 8 min.

Descriptive sensory analysis of the samples was performed by using a 10-member trained panel (all women, ranging in age between 26 and 69 years), all with previous experience in the sensory evaluation of beef. Prior to the testing phase, training was done according to the guidelines and recommendations of AMSA (2015). Sessions using physical and chemical reference standards were run during the training period so that the panelists would learn to differentiate and identify sensory descriptors, as well as to calibrate panelist on a 100-point line scale for each attribute (Table 2). Training in the intensities of aroma, flavor and texture (tenderness, juiciness) was carried out based on the study of Braghieri et al. (2012). During the training period, nine aroma, nine flavor and four textural attributes were decided upon and elucidated (Table 2). Each panel member received three pieces of beef (1 cm³ cubes) with a serving temperature of 70 °C from each LT and reference sample during training. The panelist did not detect DCP, DGP and savory broth associated with marmites for the samples during training and the attributes were therefore excluded. Panelists were allocated individual to tasting booths. The testing rooms had an environmental temperature of 21 ± 1 °C and humidity of 52 ± 2%. The permanent individual testing booths (76 cm wide × 51 cm depth) had neutral colored wall and furniture, and standard lighting conditions (700–800 lx). The testing booths were fitted with computers equipped with the Compusense five® software (Compusense, Guelph, Canada). Each animal was randomly assigned to a testing session and 8 sessions were performed (8 replications) per treatment.

2.7. Statistical analyses

All data were analyzed using the GLIMMIX procedures of SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) and standard model with diet as fixed factor and animal as random variable. Individual animal was the experimental unit. For sensory scores, the Shapiro-Wilk test was performed using PROC UNIVARIATE SAS v. 9.4 (SAS Institute Inc., Cary, North Carolina, USA) to test for normality of the residuals for each variable. Non-normal distribution, data were transformed using the Box-Cox transformation. A similar model was used for sensory analysis with diet as fixed factor, and session and panelist considered as random factors. All the data were presented in least square means (LSMEANS). The significance threshold for all statistical analyses was set at $P \leq 0.05$. To visualize the relationships between the dietary treatments and variables principal component analysis (PCA) was performed using XLSTAT software (Version 2020, Addinsoft, Paris, France) based on a Pearson's correlation matrix.

3. Results

3.1. Fatty acid composition of the experimental ingredients and diets

Diet and ingredient compositions are previously published in a companion paper by Tayengwa, Chikwanha, Dugan, et al. (2020a) and Tayengwa, Chikwanha, Gouws, et al. (2020b). For all diets, linoleic acid

Table 2

Reference standards, definition and scale of final aroma, flavor and textured attributed used for descriptive sensory analysis attributes.

Sensory attribute	Attribute description	Reference standards used
Aroma: 0 = None, 100 = Prominent		
Overall intensity	The intensity of aromas on the first few sniffs	Adapted from Braghieri et al. (2012)
Beef	The aroma associated with cooked beef steak	Roasted beef meat [Checkers, Stellenbosch, South Africa (S.A)]
Savory broth	Aroma associated with Bovril	5 ml of Bovril (Woolworth, Stellenbosch, S.A) dissolved in 250 ml boiling water
Sweet-associated	Aroma associated with the browning of a cooked meat surface (Maillard reaction)	Trimnings of roasted beef (Checkers Stellenbosch, S.A) with fat
Sour-associated	Aroma associated with sour substances (vinegar or lemon)	Acetic acid (Kimix Chemicals and Lab suppliers, Capetown, SA), 10 ppm in propylene glycol (Merck, Gauteng, SA)
Metallic	The aroma of ferrous sulfate, associated with raw meat or blood like taste	0.10% potassium chloride (Merck, Gauteng, SA) solution = 1.5 (aroma)
Fatty	Aromatics associated with cooked beef with intramuscular fat	Beef fat (Checkers, Stellenbosch, SA)
Liver-like	The aroma associated with pan fried beef ox liver	Pan fried Ox liver (Woolworth, Stellenbosch, S.A) = 7.5 (aroma)
Rancid	Aromatics commonly associated with oxidized fat and oils; may include cardboard, painty, varnish, and fishy	Microwaved Wesson vegetable oil (Woolworth, Stellenbosch, SA) for 3 min at high = 7.0 (aroma)
Flavor: 0 = None, 100 = Prominent		
Beef	The flavor associated with a cooked beef steak	Roasted beef meat (Checkers, Stellenbosch, SA)
Savory-broth	Flavor associated with Bovril	5 ml of Bovril (Woolworth, Stellenbosch, S.A) dissolved in 250 ml boiling water
Sour associated	Flavor associated with sour substances	Acetic acid (Kimix Chemicals and Lab suppliers, Capetown, SA), 10 ppm in propylene glycol (Merck, Gauteng, SA)
Metallic	The flavor of ferrous sulfate, associated with raw meat or blood-like taste	0.10% potassium chloride (Merck, Gauteng, SA) solution = 1.5 (flavor)
Sweet-associated flavor	Flavor associated with the browning of a cooked meat surface (Maillard reaction)	Trimnings of roasted beef (Checkers Stellenbosch, S.A) with fat
Salty	Taste associated with sodium ions	0.25% sodium chloride (Merck, Gauteng, SA) solution = 3.5 (flavor)
Fatty	Flavor associated with cooked animal fat	Beef fat (Checkers, Stellenbosch, SA)
Liver-like	The flavor associated with pan fried beef ox liver	Pan fried Ox liver (Woolworth, Stellenbosch, S.A) = 7.5 (flavor)
Rancid	Flavor commonly associated with oxidized fat and oils; may include cardboard, painty, varnish, and fishy	Microwaved Wesson vegetable oil (Woolworth, Stellenbosch, SA) for 3 min at high = 7.0 (aroma)
Texture		
Sustained juiciness (0 = Dry, 100 = Extremely juicy)	The amount of moisture perceived during mastication (after 5–10 chews using the molar teeth)	Adapted from Braghieri et al. (2012)
Tenderness (0 = Tough, 100 = Extremely tender)	The perceived tenderness during mastication (after 5–10 chews using molar teeth)	Adapted from Braghieri et al. (2012)

(continued on next page)

Table 2 (continued)

Sensory attribute	Attribute description	Reference standards used
Mealiness (0 = None, 100 = Abundant)	The meat disintegrates into small gritty pieces in the mouth after 5 chews	Adapted from Braghieri et al. (2012)
Residue (0 = None, 100 = Abundant)	The amount of tissue left in your mouth after mastication (after 10 chews using the molar teeth)	Adapted from Braghieri et al. (2012)

(C18:2n-6) was the major fatty acid followed by oleic (C18:1n-9) and α -linolenic (C18:3n-3) acids, respectively (Table 1). The proportions of palmitic (C16:0) and stearic (C18:0) acids were similar across diets. The proportions of C18:1n-9 and C18:3n-3 were lower in the DGP diet. The DGP diet had a lower proportion of C18:2n-6 (Table 1).

3.2. Profiles of fatty acids in beef

Intramuscular fat content was similar across diets ($P > 0.05$; Table 3). Beef fatty acid profiles were affected by the inclusion of DCP and DGP in the diet ($P \leq 0.05$, Table 3). The proportions of C18:1n-9 were greater ($P \leq 0.05$) in control-fed beef compared to DCP- and DGP-fed beef. Conversely, proportions of C18:2n-6, C20:4n-6, C18:3n-3, total conjugated linoleic acid (CLA), n-3 PUFA and total PUFA in DGP- and DCP- fed beef were 6.5%, 10.8%, 13.5%, 18.3%, 13.7% and 10.9% greater ($P \leq 0.05$) than in control fed beef, respectively.

3.3. Profiles of beef volatile compounds

A total of 44 volatile compounds were identified and the sum of each chemical class were presented in Table 4. Dietary treatments influenced the concentration of beef volatile compounds ($P \leq 0.05$; Table 4). Aldehydes (nonanal, benzaldehyde, hexanal, 2-methylbutanal and 2-methyl propanal) were found at greater ($P \leq 0.05$) concentrations in control-fed beef compared to DCP- and DGP-fed beef. Among the identified ketones, 2-butanone-3-hydroxy, 3-octanone, 2-heptanone and 2-nonanone were greater ($P \leq 0.05$) in control-fed beef than in DCP- and DGP-fed beef. Control-fed beef had greater ($P \leq 0.05$) concentrations of alcohol (2-propanol, ethyl amyl carbinol, isoamyl alcohol, 2-heptanol and 2-nonanol) than DCP- and DGP-fed beef. The DCP- and DGP-fed beef had greater ($P \leq 0.05$) concentrations of heterocyclic

Table 3

Effects of feeding diets containing dried citrus pulp (DCP) and dried grape pomace (DGP) on fatty acid composition of *longissimus thoracis* (LT) muscle from Angus steers.

Item	Treatments			SEM ¹	P-value
	Control diet	DCP diet	DGP diet		
LT intramuscular fat (mg/g muscle)	25.2	24.1	22.1	0.40	0.680
Fatty acid (% of total fatty acids)					
C18:1n-9	20.7 ^a	18.3 ^b	17.9 ^b	0.56	0.045
Σ CLA	2.5 ^b	4.8 ^a	4.7 ^a	0.21	0.003
C18:3n-3	3.1 ^b	4.8 ^a	4.8 ^a	0.42	0.012
C22:5n-3	0.4	0.7	0.6	0.47	0.102
Σ n-3 PUFA	3.5 ^b	5.5 ^a	5.4 ^a	0.70	0.025
C18:2n-6	4.9 ^b	6.0 ^a	6.1 ^a	0.59	0.031
C20:3n-6	0.5	0.6	0.6	0.62	0.214
C20:4n-6	3.4 ^b	4.8 ^a	4.7 ^a	0.43	0.023
Σ n-6 PUFA ²	8.8 ^b	11.4 ^a	11.3 ^a	0.97	0.032
Σ PUFA	13.3 ^b	18.6 ^a	18.8 ^a	0.97	0.029

^{a-b} Least squares means with different superscripts in the same row are significantly different ($P \leq 0.05$).

¹ SEM means standard error mean.

² PUFA means polyunsaturated fatty acids.

compounds (1,3-nonadiene and cyclopropane, 1-heptyl-2-methyl) and sulfur compounds (dimethyl disulfide and 3,4-dihydrothienyl (3,4, b)-5-carboxythiophene) than in control-fed beef. None of the hydrocarbons and organic acids identified were influenced ($P > 0.05$) by diet.

3.4. Profiles of sensory attributes of beef

All the aroma, flavor and texture attributes were similar across the diets (Table 5; $P > 0.05$), except for sensory tenderness. The control-fed beef was 10% more tender ($P \leq 0.05$) than DGP- and DCP-fed beef ($P \leq 0.05$). Diet had no effect on sustained juiciness, residue or mealiness ($P > 0.05$).

3.5. Relationships between fatty acid, volatile and sensory profiles of beef

The PCA variables plot of fatty acid, volatile and sensory profile of beef fed DCP or DGP is presented in Fig. 1. The first and second principal components accounted for 59.5% and 20.2% of the total variation, respectively. The control diet was clustered together with C18:1n-9, aldehydes, ketones, alcohols, sour-associated flavor, overall intensity aroma and tenderness at the center of the right quadrant. The DGP-fed beef was closely clustered with C18:2n-6, 3,4 dihydrothienyl (3, 4, b)-5-carboxythiophene), sustained juiciness, flavor (sweet associated, beef and metallic) and aroma (metallic, sweet associated, fatty and savory broth) attributes in the upper left quadrant. The DCP was closely clustered with, C20:4n-6, C18:3n-3, dimethyl disulfide, beef aroma, fatty aroma, cyclohexane, methylene and 2nonanol in the lower left quadrant.

4. Discussion

To our knowledge, the current research was the first to conduct a joint evaluation of the effects of feeding DCP and DGP on the fatty acids, volatile compounds, and sensory profiles of beef from Angus steers. The observation that the higher proportions of PUFA in meat from DCP- and DGP-fed steers included C20:4n-6 could be due to C18:2n-6 which may have undergone the processes of elongation and desaturation through the various enzymes (i.e. delta-5,6 desaturases, elongases; [Lee, Lee, Kang, & Park, 2016](#)). The increases in CLA and PUFA profiles of ruminant meat observed in the current study agree with previous studies which fed polyphenol-rich diets ([Vasta et al., 2009, 2019](#); [Guerreiro et al., 2020](#); [Mueller-Harvey et al., 2019](#)). Greater proportions of total CLA, individual and total n-3 and n-6 PUFA in DCP- and DGP-fed beef than in the control diet could be attributed to higher contents of polyphenols compounds in the former diets. Alpha tocopherol somehow modifies the biohydrogenation pathways of PUFA in the rumen, but the mechanism has not been established, and warrants investigation ([Juárez et al., 2011](#); [Zhao et al., 2013](#)). It has, however, postulated that α -tocopherol may modify ruminal biohydrogenation pathways of PUFA by acting either as an inhibitor of bacteria producing trans-10 C18:1 or as an electron acceptor for *Butyrivibrio fibriosolvens* ([Pottier et al., 2006](#)).

The presence of polyphenols in DCP and DGP diets could have elevated the proportions of total CLA, individual and total and PUFA by reducing ruminal lipolysis, and protecting PUFA from biohydrogenation in the rumen making them inaccessible to rumen microbes or their enzymes ([Vahmani et al., 2020](#); [Vasta et al., 2019](#)). Furthermore, polyphenols may interfere with the microbial cell walls, for example, through substrate deprivation and altering membrane permeability systems, hence, slow or retard growth and metabolism of rumen microbes responsible for biohydrogenation ([Luo et al., 2019](#); [Mueller-Harvey et al., 2019](#)). Proanthocyanidins, in particular, have high antioxidant activity (i.e., 50 times $>$ α -tocopherol; [Uchida et al., 1987](#)), but their availability for absorption is highly variable ([Pandey & Rizvi, 2009](#)), and their effects may in part be related to sparing of more bioavailable antioxidants such as α -tocopherol.

The increased proportions of PUFA in beef from DCP- and DGP- fed

Table 4Effects of feeding diets containing dried citrus pulp (DCP) and dried grape pomace (DGP) on profiling of volatile compounds ($\mu\text{g}/\text{kg}$) of *longissimus thoracis* muscle from Angus steers.

Volatile compounds	RT ¹	MSLM ² , %	RI ³	Treatments			SEM	P value
				Control diet	DCP diet	DGP diet		
Aldehydes								
Nonanal	20.1	72	1405	1.2 ^a	0.5 ^b	0.6 ^b	0.01	0.031
Hexanal	28.1	91	1071	5.2 ^a	2.1 ^b	1.9 ^b	0.21	0.009
Benzaldehydes	23.6	72	1520	5.7 ^a	4.2 ^b	3.9 ^b	0.01	0.034
2-methylbutanal	23.4	91	947	4.9 ^a	2.9 ^b	2.8 ^b	0.08	0.001
2-methylpropanal	21.7	88	819	4.8 ^a	1.9 ^b	1.7 ^b	0.93	0.042
Σ aldehydes				21.8 ^a	11.3 ^b	10.9 ^b	1.24	0.003
Ketones								
2-Butanone, 3-hydroxy	17.3	90	1289	5.9 ^a	2.6 ^b	2.5 ^b	1.81	0.047
3-Octanone	16.1	95	1200.3	5.4 ^a	2.8 ^b	2.3 ^b	0.88	0.037
2-Hexanone, 5-methyl	20.1	83	1300.5	0.2	0.3	0.3	0.08	0.312
2-Heptanone	13.8	91	1100.4	3.5 ^a	1.2 ^b	0.8 ^b	0.46	0.046
2-Nonanone	20.1	94	1300.5	3.4 ^a	1.9 ^b	1.7 ^b	1.07	0.016
2-Butanone, 3-hydroxy-3-methyl	16.2	83	1200.3	2.1	1.2	1.1	0.73	0.534
Acetoin (3 hydroxy-2-butanone)	17.2	90	1200.5	2.4	5.3	5.9	0.97	0.271
Σ ketones				22.9 ^a	15.3 ^b	14.8 ^b	1.03	0.031
Alcohols								
Isoamyl alcohol	14.9	90	1100.6	6.9 ^a	3.4 ^b	3.6 ^b	0.33	0.046
Isoamyl acetate	11.8	83	1000.6	1.0	0.7	0.9	0.16	0.312
2-Butanol	9.0	78	900.6	1.2	1.0	1.2	0.07	0.125
1-Butanol, 3-methyl	14.9	90	1100.7	12.3	07.4	6.6	4.14	0.203
2-Propanol	6.3	90	800.5	5.8 ^a	2.6 ^b	2.6 ^b	1.60	0.033
Ethyl amyl carbinol	20.3	90	1300.5	4.9 ^a	2.1 ^b	1.9 ^b	0.32	0.047
2-Heptanol	18.2	83	1200.6	1.3 ^a	0.3 ^b	0.2 ^b	0.10	0.036
2-Decanol	23.6	83	1400.6	0.9	0.4	0.3	0.04	0.362
2,3-Butanediol	24.2	90	1400.7	0.8	1.5	1.2	0.07	0.137
1-Hexanol, 2-ethyl	22.8	78	1084.8	1.3	1.6	2.1	0.07	0.492
2-Nonanol	23.6	83	1400.6	2.8 ^a	1.7 ^b	1.9 ^b	0.26	0.029
Σ alcohol				39.2 ^a	22.7 ^b	22.5 ^b	2.04	0.041
Heterocyclic compounds								
1,3-Nonadiene	9.7	83	900.5	10.4 ^b	12.3 ^a	12.5 ^a	0.02	0.044
Cyclopropane, 1-heptyl-2-methyl	12.2	90	1000.6	13.2 ^b	20.7 ^a	21.2 ^a	1.81	0.032
Σ heterocyclic compounds				23.6 ^b	33.1 ^a	33.7 ^a	1.63	0.041
Sulphur compounds								
Dimethyl disulfide	10.2	98	1060	8.1 ^b	14.4 ^a	13.9 ^a	0.31	0.041
Dimethyl trisulfide	19.7	96	1364	6.2	6.8	5.5	0.03	0.230
3,4-dihydrothienyl-(3,b)5carboxythiophene	8.6	83	900.2	9.3 ^b	18.3 ^a	19.1 ^a	1.20	0.044
Σ sulphur compounds				23.6 ^b	39.3 ^a	38.9 ^a	1.54	0.003
Hydrocarbon compounds								
Benzene, methyl	9.1	91	900.6	1.9	0.8	1.1	0.09	0.332
Dodecane, 1,1-difluoro	23	78	1401.0	0.4	0.2	0.2	0.03	0.267
Dichloromethane	6.1	94	800.5	0.2	0.1	1.5	0.01	0.722
Methane, bromochloro	9.4	97	900.7	1.5	1.8	2.5	0.35	0.662
Cyclohexane, methylene	13.4	87	1100.3	0.2	0.7	0.4	0.01	0.193
Cyclooctane	12.1	87	901.1	5.7	7.3	8.2	1.10	0.851
1-Undecene	12.3	97	1100.6	3.2	7.1	8.4	1.13	0.342
Trans nonene-3	12.1	90	1000.6	21.6	21.2	20.1	3.46	0.182
1-Propene, 1-(1-methoxy-1-methylethoxy)	8.6	78	900.5	4.9	3.2	3.7	0.16	0.882
Cycloheptene	13.5	93	1100.3	0.2	0.7	0.5	0.02	0.623
Tetradecane	20.2	78	1400.5	0.9	0.4	0.2	0.08	0.532
Bicyclo [4.1.0] heptane	13.7	87	1100.4	0.8	0.4	0.3	0.11	0.130
Cyclopropane, 1-heptyl-2-methyl	12.1	96	1000.6	8.1	8.3	8.2	1.34	0.782
Bicyclo [7.1.0] decane	13.5	90	1100.3	0.8	0.4	0.5	0.03	0.503
Tetradecane	20.2	78	1400.5	0.9	0.4	0.2	0.08	0.532
Σ hydrocarbon compounds				56.1	55.9	56.3	0.17	0.571
Organic acids								
Acetic acid	21.8	90	1452	1.8	4.4	4.3	0.93	0.433

^{a-b} Least squares means with different superscripts in the same row are significantly different ($P \leq 0.05$).¹ RT means retention time.² MSLM means mass spectra library match.³ RI means retention index.

steers may have led to reductions in C18:1n-9 proportions compared to that from control steers. Several studies have also reported that the proportion of PUFA in ruminant tissues is inversely related to C18:1n-9 (Hostmark & Haug, 2013, 2014; Lee, Lee, Kang, & Park, 2016). High proportions of PUFA reduce proportions of C18:1n-9 by inhibiting the delta-9 desaturase as reported previously (Hostmark & Haug, 2014; Mapiye et al., 2013; Nassu et al., 2011). Control diet might have favored the *de novo* fatty synthesis due to higher proportions of C18:1n-9 which

could have derived from C16:0 and C18:0 subjected to various elongation and desaturation processes (Vasta et al., 2019; Mueller-Harvey et al., 2019). Overall, feeding diets containing DCP and DGP to steers can promote the incomplete biohydrogenation of PUFA and yield greater proportions of n-3 and n-6 PUFA making beef more susceptible to oxidation.

Overall, the lower concentrations of aldehydes, ketones and alcohols observed for the DCP- and DGP- than control-fed beef could be

Table 5
Effects of feeding diets containing dried citrus pulp (DCP) and dried grape pomace (DGP) on sensory attributes of *longissimus lumborum* muscle from Angus steers.

Attributes	Treatments			SEM ¹	P value
	Control diet	DCP diet	DGP diet		
Aroma					
Overall intensity	76.4	76.1	76.2	0.78	0.278
Beef	69.7	70.3	70.1	0.76	0.393
Savory broth	25.6	26.1	26.3	0.69	0.779
Metallic	20.3	20.8	21.1	0.86	0.514
Sweet associated	26.2	27.1	27.3	0.76	0.562
Sour-associated	8.4	8.6	9.6	0.48	0.162
Fatty	11.9	11.9	11.9	0.61	0.996
Flavor					
Beef-like	69.9	71.1	71.3	0.74	0.467
Savory-broth	27.3	26.6	27.2	0.85	0.796
Sour associated	16.1	14.9	15.2	0.71	0.433
Metallic	25.2	24.5	26.7	0.92	0.235
Salty	11.4	10.8	11.5	0.37	0.383
Sweet-associated	24.8	25.3	25.4	0.63	0.753
Fatty	11.9	12.6	12.3	0.66	0.765
Texture					
Sustained juiciness	56.7	55.7	59.4	1.26	0.295
Residue	24.9	24.5	25.3	1.61	0.597
Mealiness	6.4	6.1	6.7	0.86	0.886
Tenderness	63.1 ^a	56.8 ^b	57.3 ^b	1.22	0.001

^{a-b} Least squares means with different superscripts in the same row are significantly different ($P \leq 0.05$).

¹ SEM means standard error mean.

attributed to higher dietary contents of polyphenols, α -tocopherol and ascorbic acid, which may have directly or indirectly reduced the rate of lipid and protein oxidation as reported in our companion study by Tayengwa, Chikwanha, Gouws, et al. (2020b), and consequently the production of their main end products (i.e., ketones, aldehydes and alcohols). Overall, the above-mentioned phytochemicals (i.e., polyphenols and α -tocopherol) have been reported to reduce levels of ketones, aldehydes and alcohols through slowing down lipid and protein

oxidation by either diminishing oxidative damage of muscle directly through activation of antioxidant enzymes or by acting as reducing agents, chelating metal ions, scavenging free radicals, quenching singlet oxygen and/or indirectly by enhancing the natural defences. (Descalzo & Sancho, 2008; Ianni et al., 2019; Vasta et al., 2019). Increased polyphenols and/or their metabolites in the tissues of steers fed fruit byproduct based diets may have also limited lipid oxidation by inhibiting the release of C20:4n-6 from phospholipids (Jenkins & Atwal, 1995).

The greater concentrations of aldehydes (i.e., hexanal and nonanal) in beef from control diet compared to beef from DCP and DGP diets is consistent with the low contents of dietary antioxidants in the former diet. Intuitively, higher PUFA proportions, due to their increased susceptibility to oxidation, would be associated with higher levels of oxidation. In the PCA, however, the control diet clustered together with C18:1n-9 and aldehydes at the center of the right quadrant, indicating greater degree of lipid oxidation in the former diet compared to DCP and DGP diets. The protective effects of antioxidants in DCP and DGP diets was also found to extend beyond lipids, as some aldehydes (i.e., benzaldehyde, 2-methyl-propanal and 2-methylbutanal) in control fed beef are likely products of amino acids (i.e., valine, isoleucine and leucine) degradation (Elmore et al., 2005; Mottram, 1998; Resconi, Escudero, & Campo, 2013).

The lack of differences among diets for hydrocarbons and organic acids is consistent with Rivas-Cañedo et al. (2013) where no effects were found when feeding polyphenolic compounds to ruminants. The observation that fruit by-product-based diets had greater concentrations of heterocyclic (i.e., 1,3-nonadiene, cyclopropane, 1-heptyl-2-methyl) and sulfur (i.e., dimethyl disulfide and 3,4-dihydrothienyl-(3,4,b)-5caboxythiophene) compounds indicate that these may have come directly from DCP and DGP diets, or as products of ruminal microbial fermentation of lignin as previously reported by Mohamed, Man, Mustafa, & Manap (2012). In addition, the higher heterocyclic compounds observed in DGP and DCP fed beef could be associated with aldehydes, which have been reported to be intermediates in the formation of these heterocyclic compounds (Domínguez et al., 2019; Elmore et al., 2005;

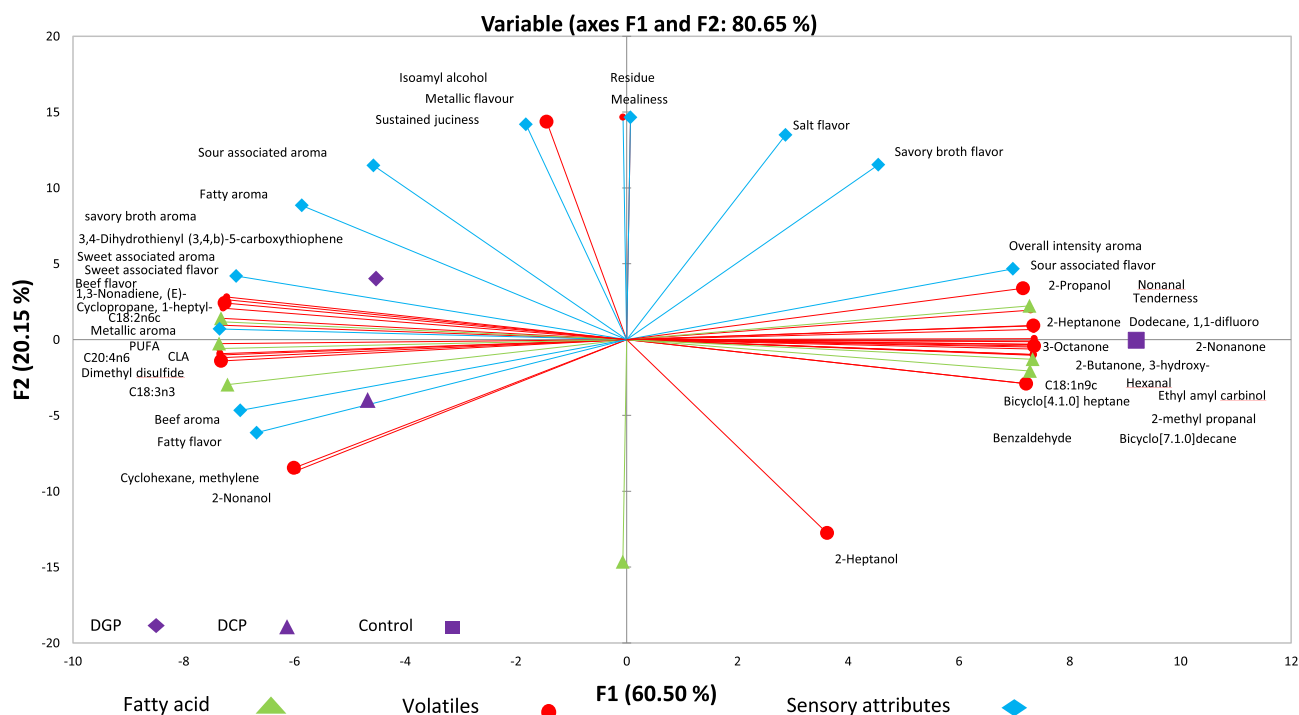


Fig 1. Variable plot obtained from principal component analysis illustrating relationships between PUFA, volatile and sensory profiles of beef fed control, DCP or DGP diets.

Mottram, 1998; Resconi et al., 2013). The presence of sulfur compounds (i.e., dimethyl disulfide and 3,4-dihydrothienyl (3,4, b)-5-carboxythiophene) in DGP and DCP fed beef could also have come from the catabolism and degradation of sulfur amino acids (i.e., methionine, cysteine and taurine) through a microbial deamination (Domínguez et al., 2019; Elmore et al., 2005; Mottram, 1998; Resconi et al., 2013).

Generally, the concentration of linear and branched volatile compounds (aldehydes, ketones, alcohol, heterocyclic, sulfur, hydrocarbons, and organic acids) detected in the present study were below threshold values known to influence sensory attributes. This could be a reason why all the cooked meat aroma and flavor were similar across diets. Furthermore, the observed similar juiciness across diets in the present study is in line with reported intramuscular fat content, drip and cooking losses reported in a companion paper (Tayengwa, Chikwanha, Dugan, et al., 2020a). For future studies, it would be recommended to compare volatile profiles of raw and cooked beef, as measurements on raw beef would provide baseline data for examining changes in flavor substances under different processing and storage conditions (Wang et al., 2017). The volatile profile of raw meat could, therefore, be used as a means of authenticating retail storage conditions and potentially meat origin (Mottram, 1998; O'Quinn et al., 2016; Xu et al., 2019).

The findings that DGP- and DCP-fed beef sensory tenderness was less than control-fed beef was consistent with instrumental tenderness (WBSF) values reported in a companion paper by Tayengwa, Chikwanha, Dugan et al. (2020a). This may be related to high contents of the observed polyphenols in the former diets, which may have directly or indirectly enhanced membrane oxidative stability during limited aging, freezing, thawing, and/or reduced early post-mortem proteolysis of beef by stimulating calpastatin, which inhibits and reduces the activity of μ -calpains (Francisco et al., 2018; Prieto et al., 2017; Tayengwa, Chikwanha, Dugan, et al., 2020a). Further research on interventions to improve tenderness in DCP and DGP fed beef may, therefore, be in order.

Overall, finishing steers on diets containing DCP and DGP increased PUFA contents and decreased concentrations of volatile compounds in beef without compromising most aspects of sensory profile as hypothesized. Our companion studies also showed that DGP and DCP cost-effectively enhanced production (Tayengwa, Chikwanha, Dugan, et al., 2020a) and shelf life extension (Tayengwa, Chikwanha, Gouws, et al., 2020b) of meat compared to the control. Overall, valorization of these fruit by-products as animal feed ingredients and natural sources of meat preservatives has potential to improve the sustainability of the feed and meat industries and decrease the cost of waste management incurred by the fruit processing industries.

5. Conclusions

Beef from DCP- and DGP-fed Angus steers had greater proportions of total CLA, individual and total n-3 and n-6 PUFA, and lower concentrations of total, alcohols, ketones and aldehydes compared to control-fed beef. Overall, the concentrations of flavor compounds were low and might explain why all the cooked meat aroma and flavor were not significantly different across diets. Feeding fruit by-products, however, resulted in less tender beef compared to control diet. Based on current findings, finishing steers on diets supplemented with citrus and winery by-products could be a feasible strategy to increase individual and total PUFA, and in beef without compromising most aspects of sensory profile. Nonetheless, additional research would be warranted to comprehensively analyse the fatty acid profile of LT and examine strategies to mitigate tenderness issues of beef fed citrus and winery by-products. Moreover, future studies are necessary to assess panel and consumer sensory profile of beef fed DCP and DGP diets during storage. Furthermore, it would be important to compare the volatile profiles of raw and cooked beef fed fruit by-products-based diets in future studies since raw beef has advantages of focusing on the changes of flavor substances under different process and storage and conditions.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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