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Untargeted Metabolomics for Beef Flavor Beyond Fat in Ground Beef With Different Lean Sources and Different Fat Content From a Common Fat Source[a]

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Abstract: To explore the effects of lean source on the flavor of ground beef patties, beef inside rounds ($n = 9$) were procured from each of the following treatment groups: commodity upper two-thirds choice (HC; USDA modest and higher marbling), heart-healthy-branded (HEART), natural grass-fed (NATURAL), and commodity USDA Select (SELECT) beef. Rounds from each source were ground and supplemented with commercially sourced, pre-ground commodity fat trim to form treatment batches containing 10% or 20% total fat. Batches were then fine-ground and formed into 113.5-g patties. Patties were vacuum packaged and frozen until analyzed. Trained sensory panel, fatty acid profile, volatile compound composition, and metabolomic features subsequently were analyzed. Lean source had no impact ($P > 0.05$) on any major trained sensory traits, but patties with 20% fat had higher ($P < 0.05$) fat-like, buttery, and juiciness scores. Patties made with HEART lean had the highest ($P < 0.05$) monounsaturated fatty acids, and those from SELECT lean had the lowest. Those patties made with NATURAL lean and 20% fat tended ($0.05 < P < 0.10$) to have higher volatile concentrations of alcohol, aldehyde, ketones, and especially terpenoid compounds. Using discriminate analysis, metabolites ($n = 64$ metabolites) from raw samples were accurately segregated by lean source only, while the cooked patties showed that the 138 metabolites were able to discriminate lean source for HC, HEART, and NATURAL within both 10% and 20% fat treatments. Patties made with SELECT lean clustered by themselves and generally had the opposite reaction to metabolite concentration as the other lean sources. Overall, while lean sources did not impact flavor, patties with different lean sources impacted the fatty acid content, volatile aroma compounds, and metabolite distribution in ground beef patties. Metabolomics may be another valuable tool to help describe meat quality, and it could be used to determine these traits in lean prior to sensory testing.

Key words: fat content, flavor, ground beef, HPLC, lean source, metabolomics
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Important findings:

- Lean sources did not impact any major sensory traits of ground beef patties.
- Monounsaturated fatty acids were highest in patties made with heart-healthy lean and lowest in pattie made with USDA Select lean.
- Ground beef patties made with natural, grass-fed beef and 20% fat had higher terpenoid volatile aroma compounds.
- Both raw and cooked metabolites metabolomics can discriminate lean source and fat content of ground beef patties.

Introduction

Flavor is the most important attribute for consumer acceptance of beef as long as tenderness is acceptable (Killinger et al., 2004; O'Quinn et al., 2012; Kerth and Miller, 2015). "Fat is flavor" is a well-known adage among the beef industry (Wasserman and Talley, 1968; Huang et al., 2010). Due to this line of thinking, the USDA quality grade system, which evaluates carcass quality based on maturity and marbling, has been a long-used tool for consumers to predict quality and for processors and retails to set prices on products. Thus, numerous studies (Corbin et al., 2014; Stewart et al., 2021) have been conducted examining marbling and fat deposition as they relate to consumer satisfaction and determining logical slaughter endpoints to increase profitability by producers. In turn, the degree of marbling has been shown to be a factor that impacts tenderness and consumer acceptability of beef products (Platter et al., 2003; Killinger et al., 2004).

In contrast, marbling has been only moderately correlated with flavor differences among carcasses from different breeds of beef cattle (Arshad et al., 2018). Research by Kerth and Miller (2015) reported that "beef identity" and "brown/roasted" flavor attributes as defined by the beef lexicon (Adhikari et al., 2011) are highly correlated to Maillard reaction products and consumer liking. The Maillard reaction, in which reducing sugars and amino acids react during high dry heat conditions, produces the browning and caramelization present in beef steaks (Dashdorj et al., 2015). However, there remains a lack of information about how particular Maillard products are produced. Recent research by Dinh et al. (2018) has shown the role of sulfur-containing amino acids, certain sugars, and nucleic compounds such as adenosine monophosphate to play a larger role in the generation of beef and brown flavor compounds than was initially thought.

Untargeted metabolomics focuses on the detection of as many groups of metabolites as possible to obtain patterns or fingerprints of biological phenomena (Cevallos-Cevallos et al., 2009). Hundreds or even thousands of metabolites can be measured, utilizing advanced chemistry detection techniques such as high-performance liquid chromatography–quadrupole time of flight (HPLC-qTOF). Statistical analyses are utilized to narrow the number of metabolites from thousands to just those that are statistically impactful on the applied treatments. This smaller number of compounds (100 to 200) may then be analyzed statistically using various multivariate approaches to then identify those influenced by applied treatments. Many pre-harvest factors have been shown to have an influence on metabolites that act as substrates in flavor-producing reactions. Specifically, Arshad et al. (2018) noted that breed variations due to genetic differences in metabolites resulted in over 40 different Maillard reaction products. The role of small sugar molecules, peptide chains, and free amino acids in the development of beef flavor is largely unknown, outside of impacts on basic tastes (Yoo et al., 2020). This leads to the possibility that the regulation of different metabolite concentrations may result in flavor differences across lean source.

In the beef industry, numerous branded programs exist based on differences in animal biological type and meat quality. These claims have led to premium products that are based on high degrees of marbling and a guaranteed tender product. Consequently, consumers would pay premium prices for these products. These claims have not been confirmed by sensory and basic meat science research. However, novel instrumentation and research methods allow us to explore the water-soluble metabolites in the lean portion of meat separate from the lipid portion (Mottram and Edwards, 1983; Huang et al., 2010). Furthermore, Legako et al. (2015) reported that the polar lipid fraction of fatty acids is largely responsible for differences in flavor as the neutral fraction undergoes little change and degradation during the cooking process, thus contributing less to flavor differences. Since the polar lipid fraction is primarily made up of phospholipids found in the muscle cell membranes and only to a minor degree in adipose tissue (Mottram and Edwards, 1983), we propose that formulating ground beef with a common fat source, but creating differential metabolomic profiles from ground beef made from differently marketed lean beef sources, would discriminate differences in flavor contribution of lean aside from adipose tissue. Defining differences in metabolites due to differences in biological type could provide evidence for differences in flavor from fatty acid profiles and up- or down-regulation of muscle metabolism. The objective of this study was to investigate differences in flavor formation in ground beef due only to the lean source by formulating ground beef with a common fat source. We hypothesize that differences in flavor and aroma compounds in the lean portion of ground beef will be the driving force for flavor differences across ground beef patty types.

Materials and Methods

Product procurement and patty formation

Beef inside rounds ($n = 9$/lean soure) were purchased from a commercial meat distributor that

advertised or qualified for 4 different lean source groups: a USDA premium branded program (upper 2/3 USDA Choice as advertised for the Certified Angus Beef Program; HC); a heart-healthy-branded program (from Akaushi cattle containing a high degree of marbling; HEART); a commodity, grass-fed all-natural program (meeting specifications for all-natural in addition to consuming a grass-based diet; NATURAL); and a USDA commodity USDA Select control group (SELECT). All rounds were processed within 7 d of packing plant fabrication and boxing. Because of the leanness of the rounds, fat content was tested for each round and found to be similar across all batches; therefore, each round was then used to make 2 batches (10% and 20% fat) of ground beef, the experimental unit was a batch of ground beef within a round, and each experimental unit was replicated 9 times. Rounds were trimmed of any visible exterior fat and ground individually (with a grinder cleanout in between rounds) using a 12.7 mm plate. Commercial, pre-ground beef fat from carcasses of grain-fed steers less than 30 mo of age was added to the ground rounds and was mixed to get an assigned targeted fat percentage (10% fat or 20% fat). Actual fat percentage was validated using the Foss FoodScan2 Meat (FOSS Global, Hilleroed, Denmark). After each batch of ground beef was formulated to the appropriate fat percentage, the blend was reground using a 4.76 mm plate. Patties were weighed and hand-formed using a handheld hamburger patty press (Oneida Hospitality Group, Lincolnshire, IL) to achieve a 113.5-g patty with a 12.7 mm thickness and 11.43 cm diameter. Six patties were formed per treatment per replication (9 replications x 2 fat contents) and were randomly designated to sensory analysis (3 patties per round per treatment), gas chromatography–mass spectrometry (GC/MS) analysis (1 patty per round per treatment), fatty acid analysis (1 patty per round per treatment), or HPLC analysis (1 patty per round per treatment). Patty paper was placed on either side of each patty, and patties were individually crust frozen at −10°C, vacuum packaged, and stored at −20°C until analysis.

Cookery and trained sensory analysis

The patties were thawed for 24 h in a cooler at 4°C for each day of the evaluation. Patties were cooked on a commercial flat top grill (Star Max 536TGF 91.4 cm Countertop Electric Griddle, Star International Holdings Inc. Company, St. Louis, MO) set at 177°C to an internal temperature of 35°C, flipped, and removed when the internal temperature reached 71°C (AMSA, 2016). Internal temperatures were monitored by iron-constantan thermocouples (Omega Engineering, Stanford, CT) inserted into the geometric center of each patty and monitored using a digital thermometer (Omega Engineering, model HH501BT type T, Stamford, CT) with a type T thermocouple (Omega Engineering, model TMQSS).

After cooking, patties were cut into 6 pie-shaped wedges. Two wedges per sample were served in 59 mL clear, plastic soufflé cups (translucent plastic 2 oz. portion cups, Georgia-Pacific, Asheboro, NC) tested to ensure that they did not impart flavors. Samples were identified with random three-digit codes and served in random order. Samples were cut and served immediately to ensure samples were approximately 37°C upon time of serving. During evaluation, panelists were seated in individual breadbox-style booths separated from the preparation area, and samples were evaluated under red lights (44.2 lux). To prevent taste fatigue, each evaluation day was divided into 2 sessions, with a 10-min break between sessions, and samples were served 4 min apart.

Human subject use was approved by the Texas A&M Human Research Protection Program prior to the study (IRB2019-0596M). Three patties (5 wedges/patty—each panelist was served 2 random wedges) were evaluated by a 5-member, expert trained beef flavor and texture descriptive attribute panel that helped develop and validate the beef lexicon. This panel was retrained using the beef lexicon for 14 d using 16-point intensity scales where 0 = none and 15 = extremely intense flavor (Adhikari et al., 2011). After training was complete, panelists were presented 12 samples per day, divided into 2 sessions. Prior to the start of each trained panel evaluation day, panelists were calibrated using one orientation or "warm up" sample that was evaluated and discussed orally. Double-distilled, deionized water, unsalted saltine crackers, and fat-free ricotta cheese were available for cleansing the palette between samples.

Fatty acid analysis

Total lipids of raw patties were extracted by a modification of the method of Folch et al. (1957). Five grams of homogenized beef was extracted in chloroform:methanol (2:1, v/v), and fatty acid methyl esters (FAME) were prepared as described by Morrison and Smith (1964), modified to include an additional saponification step described by Archibeque et al. (2005). The FAME were analyzed using an FID detector Varian gas chromatograph (model CP-3800 fixed with a CP-8200 auto sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 (100 m × 0.25 mm [i.d.]) (Chrompack Inc., Middleburg, The Netherlands), with hydrogen as

the carrier gas (flow rate = 35 mL/min; injection split ratio 20:1). Initial oven temperature was 150°C; oven temperature increased at 5°C/min to 220°C and were held for 22 min. Injector and detector temperatures were set at 270°C and 300°C, respectively. Individual fatty acids were identified using genuine external standard GLC-68D (Nu-Chek Prep, Inc., Elysian, MN).

Volatile compound analysis—GC/MS

Immediately after cooking for sensory analysis described earlier, leftover patty wedges from the sensory patties were frozen in liquid nitrogen and stored in −80°C until collection of volatile compounds. Frozen patties were powdered in liquid nitrogen, and 5 g of powdered sample was placed in a 20-mL glass vial with a Teflon lid and placed on a heating block (Block analog 2 120V with block modular 28M, VWR) held at 65°C. The volatile compounds present in the headspace were collected using a solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 µm carboxen/polydimethylsiloxane, Sigma-Aldrich, St. Louis, MO) for 20 min. Volatile aroma compounds were eluted from the SPME and separated using GC (Agilent Technologies 7920 series GC, Santa Clara, CA). The sample was desorbed at 280°C for 3 min. The sample was then loaded onto the gas chromatograph column (Agilent VF 5MS 30 m × 0.25 mm ID/1 µ film thickness, SGE Analytical Sciences, Austin, TX). Through the column, the temperature started at 40°C (held for 1 min) and increased at a rate of 20°C/min until reaching 250°C. Compounds were identified and quantified (using a 1,3-dichlorobenzene internal standard) with a mass spectrometer (Agilent Technologies 5975 series MSD, Santa Clara, CA) using the NIST/Wiley Chemical Library (Palisade, Ithaca, NY). Only compounds with total ion counts of 500 or more (about 10 ng/g) were kept for analyses.

High-performance liquid chromatography–quadrupole time-of-flight

Frozen (powdered in liquid nitrogen in a blender), 2-g samples (both raw and cooked taken from sensory patties) were placed in a 15 mL centrifuge tube containing 8 mL acidified acetonitrile (2.0% formic acid) and then centrifuged for 5 min at 4,000 × *g* (4°C). Next, 5 mL of supernate was transferred to a new tube with dSPE Enhanced Matrix Removal (Agilent Technologies; hydrated with 5 mL of water). The samples were centrifuged at 4,000 × *g* (4°C) for 3 min. The supernate was transferred to a new centrifuge tube with 3.5 g $MgSO_4$ and centrifuged at 4,000 × *g* (4°C) for 3 min. Then 200 µL of the supernate was added to a 2.5 mL sample vial with 800 µL of water. Each sample was run in duplicate using an Agilent 6545 LC/MS-QTOF using a 3.0 x 100 mm, 2.7 µm LC column (35°C; Agilent Poroshell 120 EC-C18, Santa Clara, CA) with a 1.0 µL injection volume, 0.4 mL/min flow rate in positive ion MS mode. The mobile phase consisted of acidified (0.1% formic acid) HPLC-grade water for solvent A and acidified (0.1% formic acid) methanol for solvent B with the following gradient: 0 min 97% A and 3% B; 2 min 97% A and 3% B; 8 min 0% A and 100% B; 14 min 0% A and 100% B; 15 min 97% A and 3% B. The liquid was injected into an Agilent qTOF (model 6545, Agilent Technologies, Santa Clara, CA) using dual electro-spray injection with the drying gas set at 12 L/min at 320°C, the nebulizer at 241 kpa, sheath gas flow at 11 L/min at 350°C. The mass range was 50 to 1400 *m/z* with a spectral acquisition rate of 5 spectra/s and 1553 transients/spectrum in centroid mode. Quality control samples were created by pooling equivalent volumes of all samples within a treatment, and a blank containing HPLC-grade water was used to subtract background noise. Compounds with an abundance greater than 1.0 were kept for analyses.

Statistical analyses

Trained sensory panel, volatile aroma compound, and fatty acid profile data were analyzed using ANOVA as a 4 (HC, NATURAL, HEART, or SELECT lean source) by 2 (10% or 20% fat inclusion) factorial arrangement of a completely randomized design with 9 replications, with 1 round within each lean source serving as the experimental unit and each experimental unit being subjected to both 10% and 20% fat. Lean source and fat inclusion served as fixed effects, and replication across treatments served as a random effect. Least-squares means of significant ($P < 0.05$; trends with $0.05 < P < 0.10$ were also discussed) F-tests for main and interaction effects were separated using Fisher's protected LSD (a two-sample *t*-test) using JMP Pro version 16 (SAS Institute Inc., Cary, NC).

Metabolomic data from the HPLC-qTOF were integrated using Agilent MassHunter Workstation Workflows (version B.08.00, Agilent Technologies, Inc., Santa Clara, CA). The extracted compounds were then imported into Mass Profiler Professional (version 14.9.1, Agilent Technologies, Inc., Santa Clara, CA). That subset of compounds was then filtered for those present in 75% of samples in at least one treatment, with a coefficient of variation within raw data of 25% or less,

normality with Shapiro-Wilk cutoff of 0.1, a fold-change of at least 2.0, and significance in an ANOVA ($P < 0.05$) conducted on log10-normalized data for the main or interaction effects. Compounds that were kept in the model for further analyses were identified by comparing the mass spectrum and retention time to the METLIN compound database (Guijas et al., 2018) using MassHunter ID Browser (version B.08.00, Agilent Technologies, Inc., Santa Clara, CA). Since only MS spectra were collected, we are only reporting relative differences among unidentified, untargeted compounds. Abundance values for compounds were exported to JMP where they were analyzed for hierarchical clustering and discriminate analysis.

Results and Discussion

Trained sensory panel

No interaction effects were found between lean source and fat percentage ($P > 0.05$; data not shown). Trained sensory green descriptor was higher ($P < 0.05$) in HC patties than in NATURAL patties, whereas HEART and SELECT patties were similar ($P > 0.05$) to all lean source treatments (Table 1). Textural hardness scores were higher in HEART and SELECT patties than in NATURAL patties ($P < 0.05$). Additionally, trained panel fat-like and rancid scores tended ($P = 0.075$ and 0.081, respectively) to be higher and springiness scores tended ($P = 0.069$) to be lower for NATURAL patties than in all other lean sources. Trained sensory panel scores for fat-like, burnt, buttery, heated oil, and juiciness were higher ($P < 0.05$) for patties with 20% fat than in those with 10% fat. Brown/roasted sensory scores tended ($P = 0.094$) to be higher in patties with 20% fat than in patties containing 10% fat.

We hypothesized that we would find differences in flavor and aroma compounds in ground beef patties with a common fat source but differing lean sources. However, the major trained sensory panel attributes (beef identity, brown/roasted, bloody, fat-like, and

Table 1. Least-squares means and SEM for the main effects of lean source and fat content of trained sensory panel scores of ground beef patties

	Lean source main effect						Fat percentage main effect			
Sensory[a]	HC[b]	HEART	NAT	SELECT	SEM	$P > F$	10% Fat	20% Fat	SEM	$P > F$
Beef identity	6.8	6.7	6.7	7.0	0.18	0.34	6.7	6.9	0.13	0.59
Brown/roast	8.8	8.5	8.6	8.7	0.22	0.74	8.5	8.8	0.16	0.093
Bloody	0.8	0.7	0.8	1.1	0.19	0.60	0.8	0.9	0.13	0.49
Fat-like	3.5	3.5	4.0	3.5	0.16	0.075	3.4[d]	3.9[c]	0.11	<0.001
Bitter	2.1	2.2	2.0	2.2	0.12	0.69	2.2	2.1	0.09	0.78
Salty	1.3	1.2	1.2	1.4	0.14	0.87	1.2	1.3	0.10	0.49
Sweet	0.3	0.2	0.3	0.1	0.06	0.12	0.2	0.2	0.04	0.65
Sour	2.4	2.0	2.0	2.4	0.15	0.080	2.2	2.2	0.11	0.90
Umami	4.0	3.9	4.0	4.1	0.20	0.94	3.9	4.2	0.14	0.16
Metallic	2.5	2.4	2.4	2.6	0.10	0.37	2.5	2.5	0.07	0.80
Musty earthy	0.8	0.8	0.7	0.6	0.10	0.28	0.8	0.6	0.07	0.13
Cardboardy	1.2	1.0	0.9	1.0	0.17	0.66	1.1	1.0	0.12	0.64
Burnt	0.5	0.4	0.5	0.4	0.15	0.93	0.3[d]	0.6[c]	0.11	0.047
Buttery	0.4	0.5	0.5	0.4	0.10	0.69	0.2[d]	0.6[c]	0.07	<0.001
Green	0.4[c]	0.1[cd]	0.0[d]	0.2[cd]	0.08	0.029	0.2	0.2	0.06	0.89
Heated oil	0.8	1.0	1.1	0.8	0.16	0.45	0.7[d]	1.2[c]	0.11	0.005
Liver-like	0.5	0.4	0.3	0.4	0.16	0.89	0.4	0.4	0.11	0.84
Painty	0.3[c]	0.0[d]	0.1[d]	0.0[d]	0.07	0.050	0.1	0.1	0.05	0.34
Rancid	0.0	0.0	0.1	0.0	0.04	0.081	0.1	0.0	0.03	0.31
Refrigerator	0.2	0.1	0.2	0.3	0.07	0.56	0.2	0.2	0.05	0.72
Juiciness	6.9	7.0	7.7	7.3	0.25	0.10	6.9[d]	7.6[c]	0.17	0.016
Springiness	5.2	5.5	4.9	5.4	0.17	0.069	5.3	5.2	0.12	0.41
Hardness	5.3[cd]	5.4[c]	4.9[d]	5.6[c]	0.15	0.012	5.4	5.2	0.11	0.16
Cohesiveness	5.5	5.8	5.7	6.0	0.18	0.36	5.8	5.8	0.12	0.81
Cook loss, %	29.6	28.3	27.5	27.9	1.03	0.52	26.9[d]	29.8[c]	0.73	0.007

[a]Sensory descriptor scores are on a scale of 0 to 15 with 0 = absent and 15 = extremely intense.

[b]HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.

[cd]Means in a row within a main effect category lacking a common superscript differ ($P < 0.05$).

umami) were not impacted by lean source in ground beef patties regardless of fat content. Legako et al. (2015) indicated that differences in fatty acids, and in particular fatty acids from phospholipids, were responsible for much of the differences in whole muscle strip steaks from differing quality grades. Kerth et al. (2015) reported that when ground beef patties were formulated with a common lean source, but fat sources with different fatty acid profiles, very few differences were found in consumer acceptance scores or volatile aroma compounds. It was concluded that, because a large portion of any differences in sensory attributes would be from the impact of the polar lipids found in the lean, and the lean source was common to all treatments, no differences would be found (Kerth et al., 2015). However, when both lean and fat from sources with divergent fatty acid profiles were used to make ground beef patties (Blackmon et al., 2015), differences in fatty acid profiles as well as some trained sensory panel scores (e.g., fat-like) were improved in patties that had a higher percentage of oleic acid and total monounsaturated fatty acids (MUFA). In this present study, only the green flavor descriptor significantly differed between lean sources. Surprisingly, this descriptor was perceived as higher intensity in the HC patties compared to NATURAL patties. Frank et al. (2016) similarly reported that steaks from Angus cattle finished on grain had higher intensity of grassy flavors compared to Angus cattle finished on grass-fed diets.

Fatty acid profile

Myristic acid (C14:0), palmitic (C16:0) acid, aracidic acid (20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), docosenoic acid (C22:1), and docosahexanoic acid (C22:6n-3) were not significantly impacted by lean source or fat percentage ($P > 0.05$; Table 2). Furthermore, myristoleic acid (C14:1), vaccenic acid (C18:1n-11), linoleic acid (C18:2n-6), behenic acid (C22:0), other, and total polyunsaturated

Table 2. Least-squares means and SEM for the main effects of lean source and fat content of fatty acid profiles of ground beef patties

	Lean source main effect						Fat percentage main effect			
Fatty acid[a]	HC[b]	HEART	NAT	SELECT	SEM	$P > F$	10% Fat	20% Fat	SEM	$P > F$
14:0	3.19	2.73	2.89	3.03	0.140	0.13	2.91	3.01	0.099	0.50
14:1n-5	0.53	0.55	0.49	0.51	0.041	0.39	0.58^{c}	0.49^{d}	0.029	0.031
16:0	24.84	24.66	24.51	24.51	0.094	0.054	24.55	24.71	0.068	0.093
16:1	2.37^{c}	2.27^{cd}	1.96^{de}	1.71^{e}	0.140	0.006	2.27^{c}	1.88^{d}	0.099	0.008
18:0	21.96^{d}	19.16^{e}	21.97^{d}	24.52^{c}	0.603	<0.001	20.15^{d}	23.66^{c}	0.408	<0.001
18:1n-9	37.32^{c}	38.88^{c}	36.71^{c}	34.34^{d}	0.784	0.001	37.50	36.13	0.546	0.084
18:1n-11	0.70	0.44	0.71	0.66	0.108	0.26	0.76^{c}	0.49^{d}	0.077	0.016
18:2n-6	3.66	3.41	3.66	3.49	0.212	0.78	3.82^{c}	3.29^{d}	0.150	0.014
18:3	0.29^{c}	0.22^{d}	0.23^{d}	0.28^{c}	0.285	<0.001	0.24	0.26	0.009	0.16
20:0	0.24	0.19	0.23	0.24	0.024	0.42	0.22	0.23	0.017	0.55
20:1	0.22	0.18	0.20	0.19	0.019	0.34	0.21	0.19	0.013	0.36
20:2	0.04	0.05	0.03	0.04	0.006	0.53	0.05	0.04	0.004	0.096
20:4	0.35^{d}	0.47^{c}	0.43^{cd}	0.34^{d}	0.031	0.013	0.52^{c}	0.27^{d}	0.022	<0.001
22:0	0.03	0.03	0.02	0.03	0.004	0.089	0.02^{d}	0.03^{c}	0.003	0.001
22:1	0.04^{c}	0.01^{d}	0.01^{d}	0.04^{c}	0.008	0.001	0.03	0.02	0.005	0.21
24:0	0.04^{d}	0.07^{c}	0.05^{d}	0.04^{d}	0.006	0.003	0.07^{c}	0.03^{d}	0.004	<0.001
24:1	0.09^{c}	0.05^{d}	0.04^{d}	0.08^{c}	0.009	0.001	0.08^{c}	0.05^{d}	0.006	0.001
22:6n-3	0.03	0.02	0.02	0.03	0.004	0.20	0.02^{d}	0.03^{c}	0.003	<0.001
Other	6.60	6.30	6.26	6.25	0.363	0.89	6.66	6.05	0.26	0.10
Total SFA	49.93^{d}	46.83^{e}	49.59^{d}	52.30^{c}	0.577	<0.001	47.77^{d}	51.55^{c}	0.403	<0.001
Total MUFA	41.04^{cd}	42.36^{c}	40.04^{d}	37.49^{e}	0.814	0.001	41.27^{c}	39.20^{d}	0.576	0.013
Total PUFA[2]	4.37	4.16	4.35	4.18	0.260	0.90	4.64^{c}	3.89^{d}	0.181	0.004

[a]Fatty acids are expressed as a percentage of the total fatty acids; fatty acids are defined as carbon chain length:number of double bonds with n-5, n-9, n-11, n-6, and n-3 identifying which carbon contains the double bond; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[b]HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.

[c–e]Means in a row within a main effect lacking a common superscript differ ($P < 0.05$).

fatty acids (PUFA) were not impacted by lean source ($P > 0.05$). Palmitic oleic acid (16:1) was higher ($P < 0.05$) in HC compared to either NATURAL or SE but was similar ($P > 0.05$) to HEART. Stearic acid (C18:0) was lowest ($P < 0.05$) in HEART and highest ($P < 0.05$) in SELECT compared to both HC and NATURAL, which were similar ($P > 0.05$). Oleic acid (C18:1n-9) was lowest ($P < 0.05$) in patties with SELECT lean compared to all other lean source treatments. Alpha-linolenic acid (C18:3) was higher ($P < 0.05$) in HC and SELECT compared to HEART and NATURAL, while arachidonic acid (C20:4) was higher ($P < 0.05$) in patties that used HEART lean compared to those that used either HC or SELECT. Erucic acid (C22:1) was highest in patties that had lean sourced from HC or SELECT compared to those from either HEART or NATURAL ($P < 0.05$). Lignoceric acid (24:0) was highest ($P < 0.05$) in HEART compared to all other lean source treatments, and nervonic acid (24:1) was highest ($P < 0.05$) in both HC and SELECT compared to the other lean sources. Patties with SELECT lean had the highest level of total saturated fatty acids (SFA), whereas HEART had the lowest ($P < 0.05$). Finally, patties with HEART lean had 2.3% to 4.9% more ($P < 0.05$) total MUFA compared to NATURAL and SELECT lean sources; however, HEART patties had similar ($P > 0.05$) total MUFA compared to HC.

Patties with 10% fat were 0.09% higher in myristoleic, 0.39% lower in palmitoleic acid, 3.51% lower in stearic acid, 0.49% higher in oleic acid, 0.25% higher in arachidonic acid, 0.01% lower in behenic acid, 0.04% higher in lignoceric acid, 0.03% higher in nervonic acid, and 0.01% lower in docosahexanoic acid compared to their counterparts with 20% fat ($P < 0.05$). Finally, patties with 10% fat had 3.85% lower total SFA and 2.07% and 0.75% higher levels of total MUFA and PUFA, respectively ($P < 0.05$).

Turk and Smith (2009) reported that stearic acid decreased and oleic acid was greater in ground beef from either Angus- or Wagyu-branded ground beef than in pasture-fed ground beef. Furthermore, the MUFA:SFA ratio was significantly greater in Angus and Wagyu ground beef compared to not only pasture-fed ground beef but all other commodity and fast-food ground beef. Overall, the addition of the grain-fed fat source resulted in an increase in the total SFA for all lean sources and decrease of the MUFA for all lean sources (Westerling and Hedrick, 1979). This is unfavorable from a human health perspective as unsaturated fatty acids have been shown to have more beneficial aspects compared to saturated fatty acids in food products (Lunn and Theobald, 2006; Margin et al., 2020; Djuricic and Calder, 2021). High-density lipoprotein-C (HDL-C), which is considered the "good" cholesterol, has been shown to be elevated in women (Gilmore et al., 2011, 2013) and in men (Adams et al., 2010) when they consume beef that is high in mono-unsaturated fatty acids. This is particularly true when consuming beef with high levels of oleic acid and as well as beef lower in total, saturated, and trans-fatty acids. Furthermore, in an extensive review, Smith et al. (2020) discussed producing high-oleic acid beef and beef consumption on the risk factors for cardiovascular disease.

Legako et al. (2015) reported that the percentage of MUFA increased as the USDA quality grade increased from standard to low choice to prime but decreased in cooked samples compared to raw samples regardless of quality grade. Furthermore, the percentage of PUFA from phospholipids decreased with an increase in USDA quality grade but significantly increased in cooked samples compared to raw samples, regardless of quality grade. This may indicate that the disappearance of MUFA is from their participation in lipid thermal degradation and their contribution to volatile aroma and other flavor compounds. Alternatively, MUFA could be lost disproportunately as drip loss during cooking.

Since the ground beef batches were formulated with such high percentages of fat compared to the fat inherently present from the lean source in the round, it was expected that the percentages of fatty acids would not be impacted by the lean source. There are two different theories for why differences were found. The first is that the batches formulated from HC and HEART lean contained a high enough concentration of intramuscular fat that they were not impacted as heavily by the additional fat, especially in the lower fat formulation (Lunt et al., 1993). Conversely, the additional fat source was purchased in a large amount and thus contained fat from several cattle. Therefore, the fat source itself could have been so diverse that it still led to differences between the treatment batches. Analyzing the lean sources prior to the addition of the fat and analyzing the fat separately could also help prove or disprove that theory. In future research, creating a baseline fatty acid composition from the lean source prior to formulating could help explain the differences in final composition and could help form a better theory for how fat is impacting flavor.

Volatile aroma compounds

The ng/g of volatile aroma compounds are reported in Table 3 for the main effects of lean source, fat percentage, and their interaction. In evaluating the volatiles affected by the fat% main effect, acetic acid was higher ($P = 0.002$) in patties with 20% fat than in those with 10% fat, while 1-octen-3-ol (mushroom-like aroma; Kerth and Miller, 2015) was higher in patties with HEART than in those with 20% fat ($P = 0.031$). In fact, 1-octen-3-ol was only present in 10% fat patties

Table 3. Least-squares means and SEM for the main effects of lean source and fat content of volatile aroma compounds (ng/g) of ground beef patties

									$P > F$				
	10% Fat				20% Fat					Fat% x			
Compound, ng/g	HC[a]	HEART	NAT	SELECT	HC	HEART	NAT	SELECT	SEM	Fat%	Lean	Lean	LRI
Acids													
Acetic acid	0.21	0.00	0.03	0.25	0.85	0.31	0.47	0.65	0.218	0.002	0.23	0.88	579
Alcohols													
1-octen-3-ol	0.08	0.27	0.04	0.07	0.00	0.00	0.00	0.00	0.082	0.031	0.41	0.41	983
1-pentanol	1.40^{c}	0.97^{c}	2.58^{c}	0.78^{c}	1.44^{c}	1.37^{c}	9.66^{b}	0.79^{c}	1.470	0.012	0.001	0.001	769
1-penten-3-ol	0.26^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.01^{c}	0.00^{c}	0.04^{c}	0.00^{c}	0.048	0.11	0.017	0.008	685
Aldehydes													
2-heptenal	0.00^{k}	0.01^{jk}	0.02^{j}	0.00^{k}	1.04^{k}	0.05^{jk}	0.18^{j}	0.00^{k}	0.044	0.086	0.017	0.087	964
2-methyl-butanal	4.95	5.32	0.67	3.33	7.21	10.53	11.14	5.03	2.192	0.001	0.37	0.060	665
2-methyl propanal	1.58^{cd}	2.30^{cd}	0.25^{d}	1.35^{cd}	3.20^{bc}	2.15^{cd}	5.17^{b}	2.29^{cd}	1.013	0.007	0.79	0.021	556
3-methyl-butanal	6.79^{cd}	7.26^{cd}	1.27^{d}	3.80^{cd}	10.17^{c}	10.94^{bc}	19.28^{b}	7.52^{cd}	3.294	0.001	0.44	0.012	658
Acetaldehyde	0.81^{c}	1.59^{c}	0.35^{c}	0.97^{c}	2.09^{c}	1.99^{c}	4.87^{b}	1.24^{c}	0.994	0.012	0.29	0.028	450
Benzaldehyde	1.17^{j}	0.70^{jk}	0.09^{k}	0.78^{j}	0.60^{j}	0.97^{jk}	0.47^{k}	0.46^{j}	0.291	0.74	0.048	0.18	978
Heptanal	6.46^{c}	4.60^{cd}	1.36^{d}	3.66^{cd}	6.77^{c}	5.43^{cd}	15.57^{b}	6.58^{c}	2.228	0.002	0.20	0.001	963
Hexanal	104.62^{c}	77.07^{c}	34.75^{c}	53.36^{c}	107.70^{c}	93.83^{c}	319.13^{b}	48.09^{c}	56.354	0.040	0.052	0.003	704
Octanal	2.78^{c}	2.27^{cd}	0.64^{d}	1.95^{cd}	3.54^{c}	3.00^{c}	6.95^{b}	3.15^{c}	0.886	0.001	0.30	0.001	1008
Pentanal	7.99^{bc}	4.00^{c}	2.49^{c}	3.88^{c}	1.66^{c}	3.71^{c}	15.78^{b}	0.00^{c}	3.621	0.76	0.10	0.005	702
Hydrocarbons													
4-methyl octane	0.89^{bc}	0.35^{cd}	0.02^{d}	1.21^{b}	0.37^{cd}	0.25^{d}	0.11^{d}	0.23^{d}	0.22	0.01	0.002	0.035	864
Decane	1.35^{bc}	0.74^{c}	0.13^{d}	1.55^{b}	1.00^{bc}	0.77^{c}	1.02^{bc}	0.81^{c}	0.241	0.78	0.006	0.001	999
Heptane	1.13	1.06	0.20	1.16	5.51	4.54	5.58	5.82	1.225	0.001	0.91	0.83	701
Nonane	0.30^{c}	0.29^{c}	0.06^{d}	0.42^{bc}	0.69^{b}	0.24^{cd}	0.44^{bc}	0.45^{bc}	0.107	0.006	0.021	0.035	901
Octane	2.71^{e}	3.01^{de}	0.70^{f}	2.90^{de}	6.22^{c}	4.92^{cde}	9.57^{b}	5.54^{cd}	0.978	0.001	0.49	0.001	864
Pentane	1.00	6.15	0.53	0.00	8.53	6.16	20.86	4.24	4.736	0.009	0.17	0.053	503
Ketones													
2-butanone	0.05^{k}	0.00^{k}	0.14^{k}	3.46^{j}	0.00^{k}	2.61^{k}	0.00^{k}	6.61^{j}	1.750	0.26	0.009	0.55	596
2-heptanone	0.37^{jk}	0.57^{j}	0.13^{k}	0.18^{k}	0.40^{jk}	0.44^{j}	0.25^{k}	0.18^{k}	0.143	0.96	0.031	0.79	593
2-propanone	2.23^{c}	0.77^{c}	1.40^{c}	2.08^{c}	3.79^{c}	1.22^{c}	17.72^{b}	3.03^{c}	2.621	0.005	0.001	0.001	500
2,3-butanedione	2.67^{cd}	8.76^{b}	0.30^{d}	6.36^{bc}	4.24^{bcd}	6.35^{bc}	7.68^{bc}	5.03^{bc}	2.212	0.36	0.17	0.034	587
2,3-pentanedione	0.14^{j}	0.12^{j}	0.01^{k}	0.13^{j}	0.06^{j}	0.08^{j}	0.00^{k}	0.23^{j}	0.045	0.80	0.001	0.16	699
2,5-octanedione	1.84^{k}	2.95^{j}	0.24^{k}	0.62^{k}	0.10^{k}	3.56^{j}	1.75^{k}	1.44^{k}	0.769	0.54	0.003	0.097	985
3-hydroxy-2-butanone	16.57^{k}	40.26^{j}	1.94^{k}	21.67^{jk}	24.01^{k}	56.19^{j}	37.13^{k}	39.56^{jk}	10.665	0.006	0.011	0.43	716
5-methyl-2-hexanone	0.08^{bc}	0.00^{c}	0.03^{c}	0.05^{c}	0.00^{c}	0.00^{c}	0.27^{b}	0.05^{c}	0.076	0.41	0.11	0.049	893
Pyrazines													
2,5-dimethyl-pyrazine	1.06^{k}	1.09^{j}	0.09^{k}	0.95^{jk}	0.94^{k}	3.37^{j}	0.94^{k}	1.10^{jk}	0.638	0.056	0.020	0.20	924
3-ethyl-2,5-dimethyl-pyrazine	2.20^{c}	2.30^{c}	0.59^{d}	2.41^{c}	3.14^{c}	2.84^{c}	5.28^{b}	3.46^{bc}	0.741	0.001	0.92	0.002	1087
Methyl-pyrazine	0.24^{jk}	0.36^{j}	0.04^{k}	0.25^{j}	0.33^{jk}	0.35^{j}	0.02^{k}	0.58^{j}	0.153	0.32	0.014	0.59	834
Sulfur													
Carbon disulfide	0.70^{b}	0.17^{cde}	0.06^{e}	0.40^{bcd}	0.20^{cde}	0.09^{de}	0.36^{bcd}	0.49^{bc}	0.135	0.58	0.019	0.008	541
Thiobis-methane	0.11^{k}	0.08^{k}	0.02^{k}	0.33^{j}	0.05^{k}	0.00^{k}	0.00^{k}	0.0^{j}	0.077	0.13	0.002	0.86	521

Table 3. (*Continued*)

										P > F			
	10% Fat				20% Fat					Fat% x			
Compound, ng/g	HC[a]	HEART	NAT	SELECT	HC	HEART	NAT	SELECT	SEM	Fat%	Lean	Lean	LRI
Terpenoids													
1-octene	0.17^{cd}	0.25^{cd}	0.05^{d}	0.03^{d}	0.13^{cd}	0.44^{bc}	0.74^{b}	0.19^{cd}	0.140	0.006	0.051	0.010	791
3-carene	0.21^{cd}	0.09^{cd}	0.00^{d}	0.00^{d}	0.05^{c}	0.43^{bc}	0.70^{b}	0.05^{d}	0.131	0.007	0.023	0.001	1020
Alpha-pinene	0.73^{de}	0.80^{d}	0.16^{e}	0.63^{de}	1.04^{cd}	1.60^{c}	2.68^{b}	1.03^{cd}	0.285	0.001	0.050	0.001	944
DL-limonene	0.17^{cd}	0.34^{c}	0.08^{d}	0.16^{cd}	0.28^{c}	0.74^{b}	0.78^{b}	0.29^{c}	0.088	0.001	0.001	0.001	1040
Methyl benzene	0.00^{c}	0.27^{c}	0.05^{c}	0.98^{b}	0.00^{c}	0.40^{c}	0.27^{c}	0.28^{c}	0.195	0.47	0.007	0.045	773
Toluene	1.09^{j}	1.33^{j}	0.24^{k}	0.30^{jk}	1.49^{j}	1.00^{j}	0.29^{k}	0.90^{jk}	0.439	0.52	0.020	0.69	772

[a]10% fat = calculated 10% fat and 90% lean in the ground beef; 20% fat = calculated 20% fat and 80% lean in the ground beef; HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean; LRI = linear retention index (Raza et al., 2019).

[b–e]Interaction means in a row lacking a common superscript differ ($P < 0.05$).

[j,k]Lean source main effect means in a row lacking a common superscript differ ($P < 0.05$).

and absent in 20% fat patties. Patties with 20% fat had higher levels of 2-methyl butanal (a malty, fruity arom), heptane, octane (gas-like aroma), and 3-hydroxy-2-butanone (buttery, creamy aroma; $P < 0.032$) compared to patties with 10% fat. Blackmon et al. (2015) found that as the percentage fat in ground beef patties increases, the concentration of volatiles derived from the Maillard reaction decreases. This is likely because phospholipids and their degradation products inhibit important reactions involved in the formation of heterocyclic aroma compounds in the Maillard reaction.

Patties made from NATURAL lean had slightly higher ($P = 0.017$) 2-heptenal compared to patties made with HC or SELECT but lower ($P = 0.048$) benzaldehyde (nutty, woody aroma) compared to patties made with either HC or SELECT lean. The fruity, green aroma (Kerth and Miller, 2015) of 2-butanone was by far higher in patties made in the present study with SELECT lean compared to all the other lean sources, but 2-heptanone (banana aroma) was highest for patties made with HC lean compared to those made with either NATURAL or SELECT ($P < 0.05$). The ketone 2,3-pentanedione was lowest ($P < 0.05$) for patties made with NATURAL lean compared to the other lean sources, which did not differ ($P > 0.05$). Volatiles 2,5-octanedione, 3-hydroxy-2-butanone (buttery, creamy aroma), 2,5-dimethyl-pyrazine (musty potato aroma), and methyl-pyrazine were highest in patties that were made with HEART lean compared to the other lean sources ($P < 0.05$), although volatile levels for the latter three were similar ($P < 0.05$) to patties made with SELECT. The fishy aroma thiobis-methane was highest in patties made with SELECT lean compared to the other three sources, and toluene was lower in patties made with NATURAL lean compared to either HC or HEART ($P < 0.05$).

1-pentanol was significantly higher in NATURAL patties with 20% fat, while 1-penten-3-ol (grassy/green aroma; Burdock, 2009) was higher in HC 10% patties compared to all other fat% and lean source combinations ($P < 0.05$). All 7 of the aldehyde volatiles significant for a fat% by lean source interaction were higher in patties made with 20% fat and the NATURAL lean source ($P < 0.03$). However, patties made with 20% fat and HEART lean were similar ($P > 0.05$) to the 20% NATURAL patties in 3-methyl-butanal (malty), and both heptanal (fatty, harsh aroma) and octanal (citrus-like) volatiles were lower ($P < 0.05$) in 10% fat NATURAL patties compared to 10% fat HC patties. The volatile 4-methyl-octane was higher ($P < 0.05$) in patties with 10% fat and SELECT lean except 10% HC patties, which were similar ($P > 0.05$).

Dimethyl disulfide (onion; Burdock, 2009) was higher in HC and HEART than in NATURAL patties ($P < 0.05$). Patties with 10% fat and SELECT lean along with HC and NATURAL patties with 20% fat are higher in the volatile decane compared to 10% HEART, 20% HC, and 20% SELECT patties, and 10% NATURAL was the lowest compared to all other treatment combinations ($P < 0.05$). Lean source did not impact ($P > 0.05$) nonane within the patties made with 20% fat, but patties made from NATURAL lean was the lowest ($P < 0.05$) within those patties with 10% fat. Interestingly, octane is lowest for patties made with NATURAL lean within those with 10% fat and is the highest for patties made with NATURAL lean within those patties with 20% fat. The volatile 2-propanone (acetone) was unaffected by lean source within the

10% fat patties, but patties made with NATURAL lean with 20% fat was 13.93% to 17.00% higher than all other treatment combinations ($P < 0.05$). The buttery-smelling volatile 2,3-butanedione was unaffected ($P > 0.05$) by lean source within patties with 20% fat, but within patties with 10% fat those with HEART lean were higher and NATURAL lean were lower ($P < 0.05$). Patties with 20% fat and NATURAL lean had the highest ($P < 0.05$) 5-methyl-2-hexanone compared to others, except for patties with 10% fat and HC lean ($P > 0.05$). The caramel/coffee-smelling volatile of 3-ethyl-2,5-dimethyl-pyrazine was highest for patties made with NATURAL lean and 20% fat and lowest for those patties with NATURAL lean made with 10% fat ($P < 0.05$). Patties with 10% fat had higher ($P < 0.05$) carbon disulfide volatile in patties made with either HC or SELECT, while patties with 20% fat had higher ($P < 0.05$) carbon disulfide in patties with SELECT lean compared to those with HEART lean. The terpenoids 1-octene and 3-carene had similar ($P > 0.05$) concentrations for all lean sources within the patties made with 10% fat, while patties made with 20% fat had higher ($P < 0.05$) levels in patties made with NATURAL lean compared to those made with HC or SELECT. Alpha-pinene was similar ($P > 0.05$) among lean sources in 10% fat patties, but in patties with 20% fat and NATURAL lean were higher ($P < 0.05$) than the other lean sources. The lemon-like DL-limonene volatile was lowest in the NATURAL lean patties with 10% fat, while patties with HEART and NATURAL lean within 20% fat had higher levels compared to patties made with either HC or SELECT ($P < 0.05$). Finally, patties made with HEART lean and 10% fat had the highest levels of methyl-benzene compared to all other treatment combinations ($P < 0.05$).

It is not surprising that the NATURAL patties exhibited more differences in volatile aroma compounds, as this was the only treatment that claimed on the label to be finished on a grass-based diet. It is assumed that cattle for all other treatments were finished on a concentrate diet. Additionally, 2-heptenal could also be a contributing factor to the grassy flavor as it has been described as herbaceous. Dimethyl disulfide was found in greater concentrations in patties made with lean from higher USDA quality grades. Gardner and Legako (2018) reported the highest concentration of dimethyl disulfide in USDA Prime steaks and decreasing concentration with decreasing quality grades. Therefore, dimethyl disulfide could be partially responsible for increase in beefy, brown/roasted flavors that drive consumer liking of premium branded projects. Mottram (1998) indicated that sulfur-containing compounds formed in the Maillard reaction from sulfur-containing amino acids like cysteine and reducing sugars like ribose (from inosine monophosphate and other ribonucleotides) seem to be particularly important for the characteristic aroma of meat. Elmore and Mottram (2006) reported greater concentrations of 2-octene, an isomer of 4-octene in cattle finished on a silage diet. Additionally, they reported that cattle fed on concentrate diets had greater concentrations of 1-octen-3-ol and pentanal compared to silage finished cattle.

Mottram (1998) did a thorough job of detailing the formation of flavor in meat, noting the water-soluble products from the Maillard reaction as well as the lipid-derived volatiles that are a result of lipid thermal degradation. He also noted that these two categories of volatile compounds do not act independently of each other and that, in particular, lipid oxidation products like aldehydes and carbonyls react readily with Maillard-derived intermediates. This gives rise to additional aroma compounds and may limit those compounds normally seen in the Maillard reaction. The current research does not necessarily follow this theory as many of the lipid-derived compounds like aldehydes, alcohols, and acids were found in higher concentrations in those patties that had 20% fat, and many of the Maillard products like the pyrazines increased in the patties with the higher fat percentage, but then some products like the sulfur-containing compounds and terpenoids were lower.

Numerous investigators have reported on the resulting differences in flavors and volatiles resulting from beef fed different diets (Melton, 1990, 1999; Maruri and Larick, 1992; Cox et al., 2006; Kerth et al., 2007). In particular, Maruri and Larick (1992) found that terpenoids and long-chain hydrocarbons were moderately and positively correlated with a gamey/stale off-flavor found in ground beef made from cattle that have been finished on grass. Brown et al. (1979) reported that ground beef from steers fed on forage had higher amounts of free fatty acids and that steers fed grain had higher free sugar content compared to their forage-fed counterparts. In general, cattle that are finished on grain diets induce higher levels of branched-chain fatty acids, some aldehydes, and lactones, whereas cattle finished on grass tend to contain higher levels of phenols, terpenes, indoles, and sulphur compounds (Vasta and Priolo, 2006). As possible precursors, Koutsidis et al. (2008) found that animals fed grass had higher levels of free amino acids, whereas those fed concentrates had a higher total reducing sugar content.

HPLC—Metabolomics

Zhang et al. (2021) described the workflow of metabolomics analyses that included biological questions, sample preparation, metabolomics analyses, and data interpretation. In interpreting data, the two major steps are data preprocessing or pretreatment and biological interpretation. Due to the complexity of the data as well as the relatively large data sets, multivariate analyses have been the most chosen statistical pathway. This includes partial least squares discriminate analysis, principal component analysis, and machine learning. We will report hierarchical clustering and discriminate analyses.

Ground beef patties were analyzed using HPLC-qTOF for untargeted metabolomics in both the raw (Figures 1 to 3) as well as the fully cooked (Figures 4 to 6) state. A total of 64 molecules were significantly affected ($P < 0.05$) by treatment combinations in raw ground beef samples. A two-way hierarchical cluster analysis for these metabolites is illustrated in Figure 1. Different colored bars represent differences in the $\log_{10}$ relative abundance of each metabolite across each treatment combination. The raw cluster is the presence of 4 different compounds that are likely carnitines which have been correlated to consumer acceptance, tenderness, and juiciness (Antonelo et al., 2020a, 2020b). Hierarchacal clusters using the Ward method were identified both across treatment combinations but also across metabolite. Whereas the patties with either 10% or 20% fat clustered together across HEART, HC, and NATURAL lean sources, both the heat map as well as the constellation plot (Figure 2) shows that SELECT patties with 20% fat clustered with the remaining 10% fat lean sources and vice versa for the SELECT patties with 10% fat. Using partial least squares discriminate analyses (Figure 3), treatment combination means with 95% confidence interval ellipses and 50% contours showed that only the lean source main effect accurately clustered metabolites into the 4 lean source categories.

The cooked patty two-way hierarchical cluster analyses (Figure 4) showed 3 main treatment clusters. Again, patties with both 10% and 20% fat in the SELECT lean source clustered together and apart from the other treatment combinations. The remaining lean sources with 10% fat and those with 20% fat each clustered together. Furthermore, the metabolites (as listed in Table 4) appeared to cluster into 3 primary clusters across the treatment combinations. These 3 treatment combination clusters are also illustrated in the constellation plot found in Figure 5. Interestingly, when looking at the fatty acid profile (Table 2), it appears that the percentage of oleic acid parallels the metabolites from the cooked patties. Perhaps many of the metabolites may be

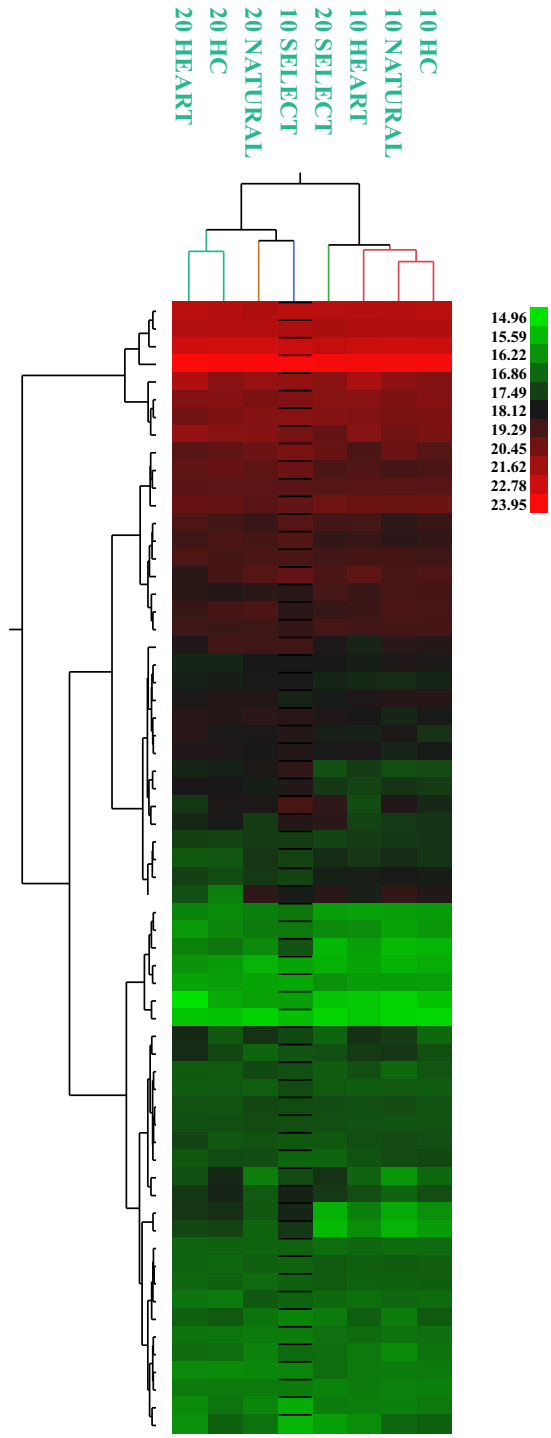


Figure 1. Two-way hierarchical cluster analysis of 64 raw small molecule metabolites. Each color bar represents the $\log_{10}$ relative abundance of each annotated metabolite. Those molecules not identified are expressed as mass:charge (m/z) @ the column retention time. 10 = 10% fat ground beef patties, 20 = 20% fat ground beef patties, HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.

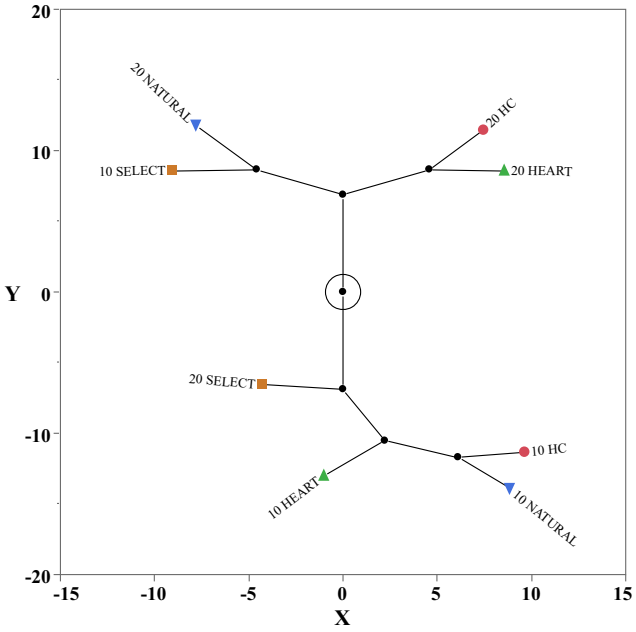
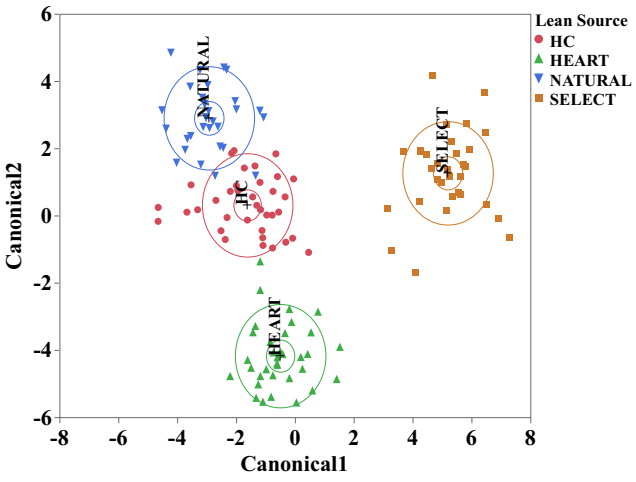


Figure 2. Constellation plot for 64 raw small-molecule metabolites. 10 = 10% fat ground beef patties, 20 = 20% fat ground beef patties, HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.



Training

Actual Lean Source	Predicted Count HC	HEART	NATURAL	SELECT
HC	36	0	0	0
HEART	0	34	0	0
NATURAL	0	0	31	0
SELECT	0	0	0	32

Figure 3. Partial least squares discriminative analysis of 64 raw small-molecule metabolites sampled from ground beef with four different sources of lean with 95% confidence ellipses and 50% countours around each treatment mean. HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.

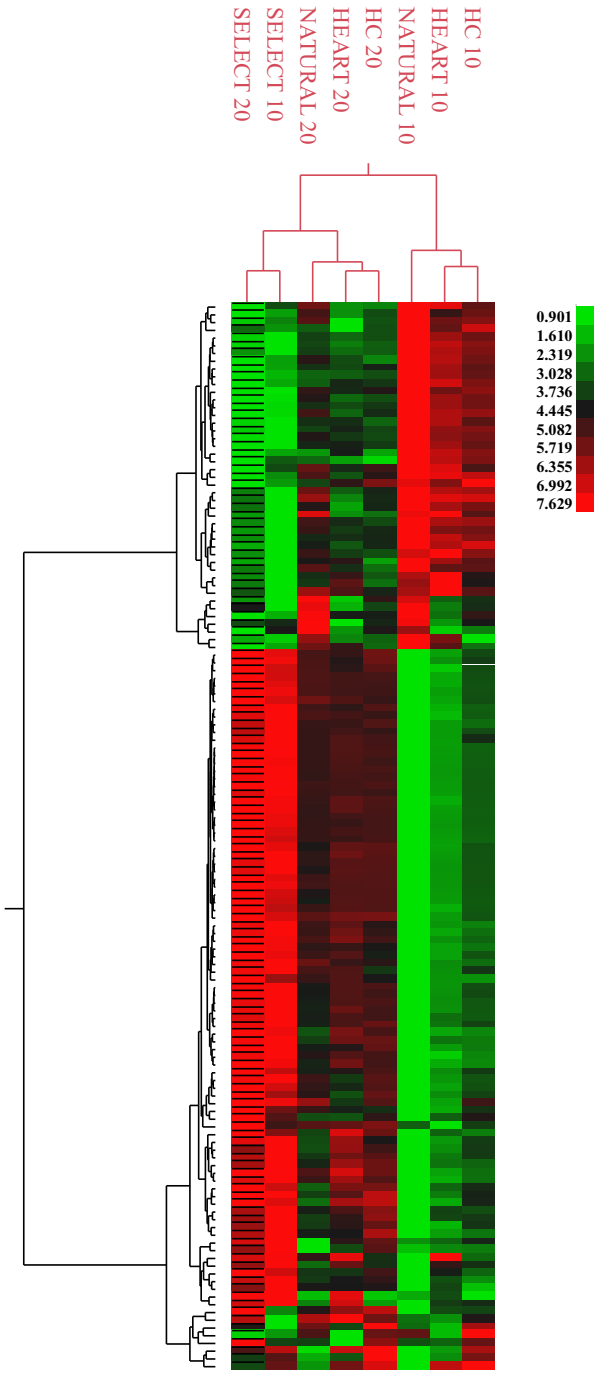


Figure 4. Two-way hierarchical cluster analysis of 138 cooked small-molecule metabolites. Each color bar represents the $\log_{10}$ relative abundance of each annotated or unidentified metabolite. Those molecules not identified are expressed as mass:charge (m/z) @ the column retention time. 10 = 10% fat ground beef patties, 20 = 20% fat ground beef patties, HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.

regulating the proportions of fatty acids in the ground beef. Several investigators (Kim et al., 2018; Setyabrata et al., 2021, 2022) have used metabolomics to describe the flavor precursors and flavor changes as a result of aging, and Jeong et al. (2020) even described the impact of specific carnitines present in highly marbled beef that had high sensory scores. The discriminate analysis showed very tight clustering of treatments for cooked ground

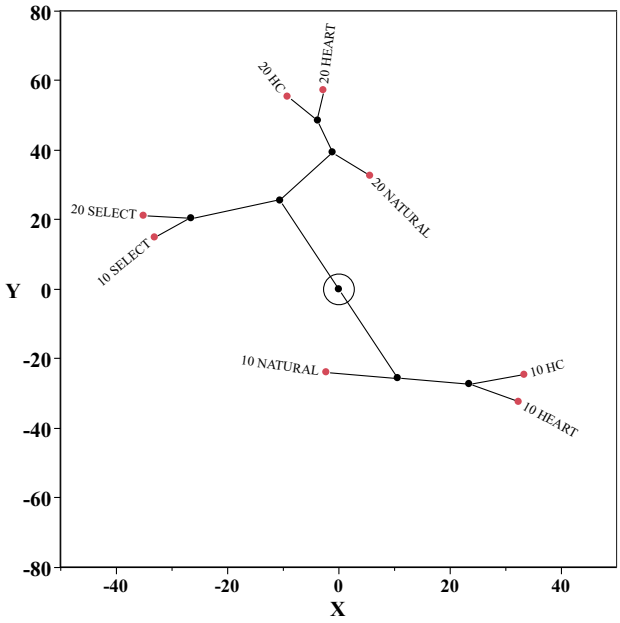
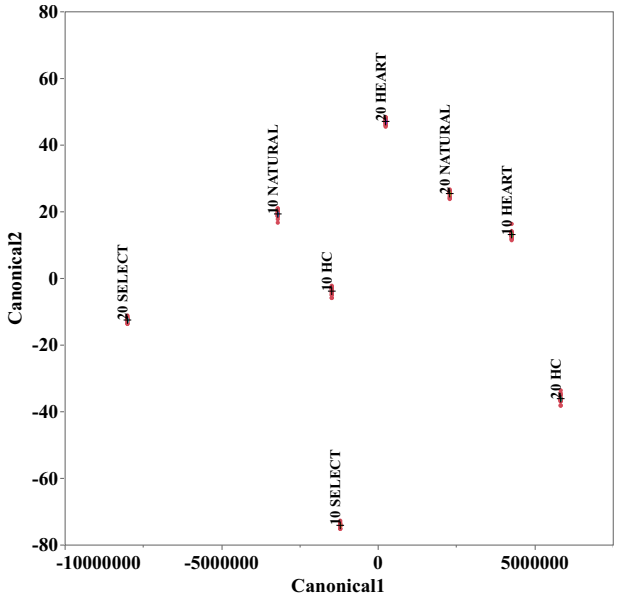


Figure 5. Constellation plot for 138 cooked small-molecule metabolites. 10 = 10% fat ground beef patties, 20 = 20% fat ground beef patties, HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.



Actual Fat Lean	Predicted Count 10 HC	10 HEART	10 NATURAL	10 SELECT	20 HC	20 HEART	20 NATURAL	20 SELECT
10 HC	18	0	0	0	0	0	0	0
10 HEART	0	18	0	0	0	0	0	0
10 NATURAL	0	0	19	0	0	0	0	0
10 SELECT	0	0	0	13	0	0	0	0
20 HC	0	0	0	0	17	0	0	0
20 HEART	0	0	0	0	0	18	0	0
20 NATURAL	0	0	0	0	0	0	15	0
20 SELECT	0	0	0	0	0	0	0	14

Figure 6. Partial least squares discriminative analysis of 64 raw small molecule metabolites sampled from ground beef with four different sources of lean with 95% confidence ellipses and 50% countours around each treatment mean. 10 = 10% fat ground beef patties, 20 = 20% fat ground beef patties, HC = high choice lean; HEART = heart-healthy-branded lean; NAT = naturally branded lean; SELECT = USDA Select lean.

Table 4. List of likely annotated small metabolite compounds or unknown (m/z @ retention time) from raw and cooked ground beef samples. Each compound that was found matches a colored bar across all fat percentage and lean source treatments representing the $\log_{10}$ relative abundance of each compound in the heat map found in Figures 1 and 4.

Raw metabolomic compound	$n = 64$
Unknown	25
Metabolites	18
Amino acids/peptides	5
Carnitines	5
Organic acids	7
Fatty acids/derivatives	4

Cooked metabolomic compound	n
Unknown	89
Metabolites	22
Amino acids/peptides	10
Carnitines	4
Organic acids	4
Fatty acids/derivatives	3
Heteroaromatic compound	3
Hormones	3

beef patty small-molecule metabolites across all fat content by lean source interaction means.

Researchers have shown that beef high in MUFA, and especially oleic acid, is correlated to positive trained sensory and consumer acceptance scores (Blackmon et al., 2015; Kerth et al., 2015; Listrat et al., 2020). There is some discussion that the source of these positive fatty acids may be sourced in the polar phospholipids of the lean cell membrane and to a lesser extent in the lipids from adipose tissue (Legako et al., 2015; Hunt et al., 2016). This would support the interaction of lean source and fat content found in the present study as fatty acid differences were found among lean sources in the 10% fat patties, but it seems that the added fat in the 20% fat patties diluted the MUFA and palmitoleic acid and oleic acid in particular. It should be noted that the MUFA content of the SELECT 10% patties was similarly low as that found in all of the 20% fat patties. When evaluating the small-molecule metabolite clustering in both raw (Figure 1) and cooked (Figure 4), HEART, NATURAL, and HC all three cluster together in the 10% fat patties. Furthermore, in the cooked heat map, 10% and 20% fat SELECT ground beef patties cluster together and generally have opposite reactions in the metabolites compared to all other treatment combinations.

Jiang and Bratcher (2016) reported 22 metabolic compounds that were significant in a principal components analysis of metabolites from ground beef sourced from different lean sources. In their analysis, they demonstrated that grass-fed beef differentiated from natural beef and that oleic acid was identified as one of the important compounds influencing beef flavor. Ma et al. (2017) found that acyl carnitines were dominant in the *psoas major* muscle compared to the *longissimus lumborum* or *semimembranosus* and in muscles that were aged 9 d compared to those aged 16 or 23 d. They also reported that L-carnitine was negatively correlated to meat color traits during display. They hypothesized that higher levels of carnitine may indicate a higher level of energy metabolism and mitochondrial enzyme activity in antemortem muscles. This condition may cause an increase in the oxygen consumption rate of aged muscle and eliminate free oxygen penetrating the muscle and reacting with myoglobin, resulting in a dark purplish color from the presence of deoxymyoglobin.

Cho et al. (2017) demonstrated a positive relationship between short-chain fatty acid-carnitine (e.g., proprionyl carnitine) levels and insulin resistance. Other research (Koves et al., 2005) indicated that rats that were fed high-fat diets throughout the study had consistently higher levels of acylcarnitine levels, particularly proprionyl, butanoyl, and hexanoyl carnitine, whether fasted or fed, indicating the inability of the muscle to metabolize short-chain fatty acids and amino acids with beta-oxidation. In a study comparing Korean cattle with low marbling (11.6% lipid) and high marbling (16.9% lipid), NMR analysis showed that carnitine along with other metabolites like creatine, lactate, and carnosine increased in longissimus muscle from high marbled beef (Jeong et al., 2020). Conversely, Mateescu et al. (2012) reported that when they tested over 2,000 head of Angus cattle, not only did carnitine not correlate with any sensory panel descriptive ratings, but carnitine was lowly heritable ($h = 0.015$). It is worth considering in the latter case whether the low heritability coefficient is due to a relatively homogeneous group of cattle within one breed. It would be interesting to see whether heritability would change with an increase in the variability of genetics.

Conclusions

While the lean source and fat content of the ground beef patties used in this study had little impact on trained sensory panel scores, several differences in fatty acid profiles, volatile aroma compounds, and non-volatile flavor precursors and flavor compounds were found. It was particularly interesting to see that both raw and cooked samples analyzed by metabolic profiling had compounds that contributed to differences in ground beef lean and fat content treatments. Certainly, metabolic factors and(or) small molecules in the raw and cooked patties may be those that impact traditional meat quality parameters, and additional work to positively annotate those compounds is necessary. It appears that with these metabolomic findings, future research should focus on correlating these compounds with sensory and flavor traits. The ultimate goal, in considering the entire production system from growth to consumption, would be able to track these metabolites further back in the production of beef that would allow us to be able to determine future beef quality. This could include sampling serum from cattle destined for harvest to determine quality and sensory traits.

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