METABOLOMICS OF COOKED GRASS- OR GRAIN-FED BEEF STEAKS DIFFERING IN MARBLING CONTENT AND COUNTRY OF ORIGIN

Kayley R. Wall[1*], Chris R. Kerth[1] and Rhonda K. Miller[1]
[1]Texas A&M University, College Station, TX 77843, USA;
*Corresponding author email: wall4165@tamu.edu

I. INTRODUCTION

Many factors influence beef flavor, and arguably one of the greatest influencers is the diet of the animal [1,2]. Sensory panel, volatile aroma compound, and fatty acid differences have been detected in grass- and grain-finished beef animals [3,4]; however, the non-volatile fraction of cooked beef has not been evaluated. The objective of this study was to analyze the non-volatile component of commercially-available, cooked beef strip loin steaks that vary in diet source, marbling content, and country of origin.

II. MATERIALS AND METHODS

Beef strip loin steaks (2.54 cm; n = 23) were purchased from a local retail store. Steaks were imported Australian grass-fed (AUS; n = 6), conventional USDA grade upper 2/3 Choice grain-fed (Choice; n = 6), name-brand high-marbled grass-fed (High Grass; n = 6), and name-brand low-marbled grass-fed (Low Grass; n = 5) beef. Steaks were kept in cold storage (4°C) in retail packaging until testing day. Steaks were cooked on an electric flat-top grill to 71 °C. Cooked pieces (1.3 cm x 1.3 cm x steak thickness) were quick-frozen in liquid nitrogen. Frozen samples were homogenized in a blender and 2g were placed in a 15 mL centrifuge tube with 8 mL acidified acetonitrile (0.1% formic acid) and then centrifuged for 5 min at 4,000 x *g* (4 °C). Next 5 mL of supernatant 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 x *g* (4 °C) for 3 min. The supernatant was transferred to a new centrifuge tube with 3.5 g $MgSO_4$ and centrifuged at 4,000 x *g* (4 °C) for 3 min. Then 200 µL of the supernatant 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 (Table 1). Data were analyzed using MassHunter Qualitative Workflow, Profinder, and Mass Profiler Professional software (B.08, Agilent Technologies). Compound groups were filtered using the following criterion: absolute peak height of 2000 relative intensity, retention time window of 0.3 min, and molecular feature extraction score of greater than 80. Only those compound groups present in 5 or more of the samples in a treatment set were analyzed. Data were analyzed using analysis of variance with treatment as fixed effect and alpha set at 0.05. A moderated t-test and Tukey's least squares means was utilized to determine differences in fold changes among pairs of fixed effects.

Table 1. LC/MS-QTOF Parameter Settings

LC Parameter	LC Setting	MS Parameter	MS Setting
LC column	Agilent Poroshell 120 EC-C18 3.0x100mm, 2.7-micron	Drying Gas Flow	12 l/min at 320 °C
Injection Volume	1.0 µL	Nebulizer	35 psig
Flow Rate	0.4 mL/min	Sheath Gas Flow	11 L/min at 350 °C
Thermostat Column Temperature	35.0 °C	VCap	3,500 V
Ionization Mode	Positive mode, MS	Mass Range	50 – 1,400 *m/z*
Mobile Phase	A) Water, 0.1% formic acid B) Methanol, 0.1% formic acid	Spectral Acquisition Rate	5 spectra/s, 1,553 transients/spectrum, centroid mode
LC Gradient	Time A (%) B (%) 0 97 3 2 97 3 8 0 100 14 0 100 15 97 3		

III. RESULTS AND DISCUSSION

Non-volatile compound groups (n = 878) were determined to be present in AUS (n = 853), Choice (n = 795), Low Grass (n = 850) and High Grass (n = 802) steaks. Similar and unique compound groups were identified among steak treatments with 87.2% of the total compound groups in common, as summarized in Figure 1. AUS and Low Grass shared the greatest number of compound entities and were more similar in grouping due to levels of intensity of compound abundances (Figure 2). Accordingly, Choice and High Grass conditions separated from AUS and Low Grass conditions on the first principal component with 82.75% of the variation accounted for (Figure 3).

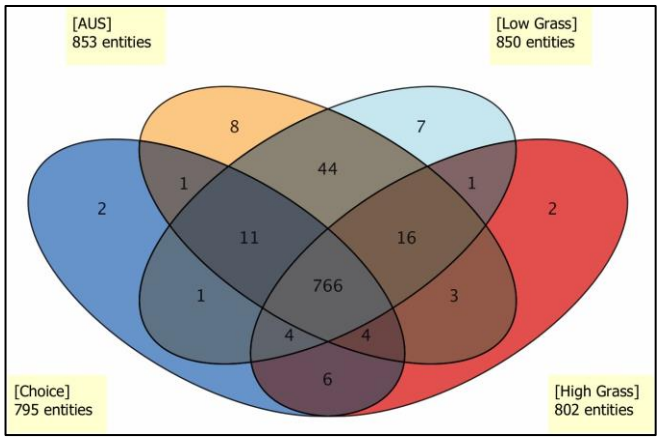

Figure 1. Unique compound groups among cooked, commercially available imported AUS, Choice, and Low Grass and high marbled High Grass beef steaks.

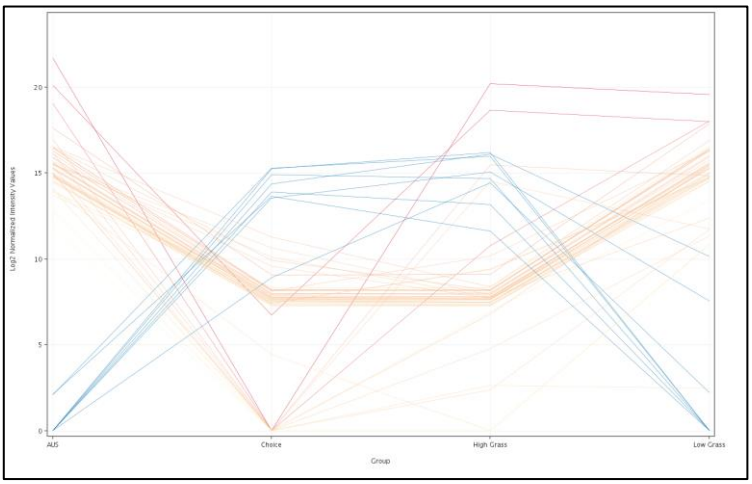

Figure 2. Profile plot of strong influencing (Pearson's |r| > 0.85; |covariance| > 0.95) non-volatile compounds (n = 55). Compound entities are plotted by relative intensity values and colored by high (red), moderate (orange), and low (blue) levels of abundance in the AUS category.

IV. CONCLUSION

The analysis of cooked meat samples depicted similarities of non-volatile compound groups which stratified beef strip steaks. Commercially purchased name-brand low-marbled beef was similar to imported Australian beef strip steaks; whereas, name-brand high-marbled beef was similar to conventionally fed USDA Choice steaks. Accordingly, animal diet and product marbling similarities can be observed and may have a stronger association than animal origin or genetics in the non-volatile fraction of cooked beef steaks.

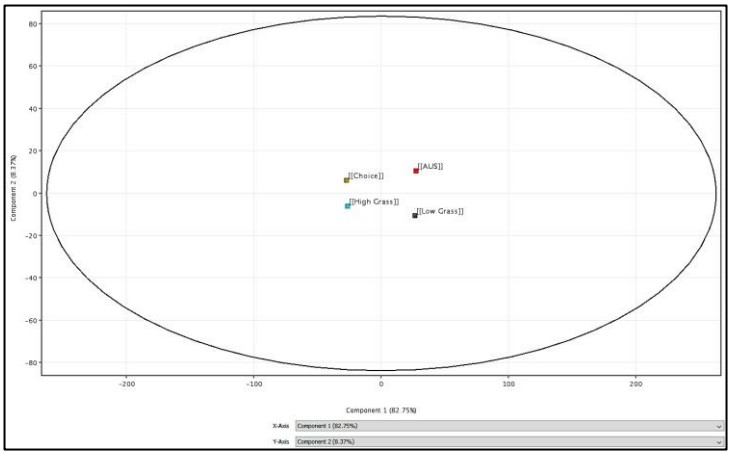

Figure 3. Principal component analysis (PCA) of cooked, commercially available imported AUS, Choice, and Low Grass and high marbled High Grass beef steaks.

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