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Effects of unsaturated fatty acids, lipid oxidation, myoglobin, and hemoglobin on livery flavor volatiles in beef steaks

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EFFECTS OF UNSATURATED FATTY ACIDS, LIPID OXIDATION, MYOGLOBIN, AND HEMOGLOBIN ON LIVERY FLAVOR VOLATILES IN BEEF STEAKS[1]

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Summary

Infraspinatus (top blade), gluteus medius (top sirloin), and psoas major (tenderloin) steaks were obtained from A- and B-maturity carcasses that had either a high (≥ 6.0) or normal (≤ 5.7) pH, and either slight or small marbling. The steaks were vacuum aged until either 7, 14, 21, or 35 days postmortem. The steaks were broiled and served to a highly trained flavor-profile sensory panel. Steaks with livery flavor were subjected to gas chromatography/mass spectrometry analyses for flavor compounds. Steaks aged until 7 or 35 days postmortem were analyzed for the 2-thiobarbituric acid reactive substances (TBARS) content to determine lipid oxidation and for myoglobin and hemoglobin concentrations. Thirteen different volatile compounds had greater amounts in steaks with livery flavor. Lipid oxidation of raw steaks was not related to livery flavor, but steak myoglobin concentration was related to livery flavor.

Introduction

Livery flavor is an off-flavor sometimes associated with beef. Livery flavor is objectionable to many consumers, and meat purveyors have received numerous complaints. Little research has been conducted on the volatiles responsible for livery flavor. Limited research has been conducted to determine if myoglobin and hemoglobin concentrations are related to the development of livery flavor. Therefore, the objectives of our study were to determine if pigment concentration, lipid oxidation, and fatty acid composition were related to livery flavor and to identify the volatile compounds that were related to livery flavor. This project was funded with Beef Checkoff dollars and coordinated by the National Cattlemen's Beef Association.

Experimental Procedures

Subprimal Selection. Beef chuck, shoulder clods; loin, top sirloin butts; and loin, full tenderloins were obtained from two commercial beef slaughter and processing facilities at six different sampling times. The infraspinatus, gluteus medius, and psoas major muscles were excised from each of the respective subprimals. Carcasses were selected to fit into two groups: 1) carcasses of A-maturity and 2) carcasses of B-maturity bone. These groups were further selected to be of two pH subgroups: 1) those having an ultimate pH of 5.7 or less and 2) those having a pH of 6.0 or greater (dark cutters). The carcasses also were

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selected to be of two marbling groups: 1) those having USDA marbling scores of Slight^{00} to Slight^{50} and 2) those having USDA marbling scores from Small^{00} to Modest^{00}. The subprimals were fabricated at 7 days postmortem, and the individual muscles of interest were removed from each subprimal. Steaks 1-inch thick were cut from the muscles, randomly assigned to an aging treatment (7, 14, 21, or 35 days), and vacuum packaged. Steaks were aged at 36 to 39°F until either 14, 21, or 35 days postmortem. All steaks were then frozen and stored at -40°F until analyses.

Quantification of Myoglobin and Hemoglobin. HPLC analyses were conducted on pulverized steak samples with a Hewlett-Packard Series II, 1090A HPLC. A 50 μL buffered volume of the filtered sample was injected into the HPLC instrument. Horse skeletal muscle myoglobin, bovine hemoglobin, sodium phosphate, and ammonium sulfate were used to make stock solutions containing varying levels of myoglobin and hemoglobin. The solutions were prepared using a 0.1 M sodium phosphate buffer, and these solutions were used to prepare standard curves.

Gas Chromatography/Mass Spectrometry. Steaks were cooked on a Wells B-44 charbroiler. Cooked samples that were found to be livery by the highly trained descriptive-flavor-profile sensory panel were frozen for later gas chromatography. Samples were divided into groups of either "extremely" livery (>2.0 on 15-point sensory scale), "moderately" livery (0.5-1.5), or control (0). These samples were frozen and used to identify which compounds contributed to livery flavor and to determine if amounts of some compounds increased as livery flavor increased.

Ten grams of frozen pulverized sample plus 40 mL of distilled water were steam distilled until a total of 8 mL had accumulated in a glass test tube. The distillate was then poured into a glass headspace vial, capped, and frozen until thawed for analysis. The samples were then placed in a water bath at 176°F and a 100 μm poly-dimethyl-sulfoxide fiber (Supelco, Bellefonte, PA) was inserted into the vial. The fiber was exposed to the headspace in the vial for 20 minutes. The fiber was then removed from the vial, and inserted through a septum into the Hewlett-Packard 5890 gas chromatography/mass spectrometer.

TBARS Analyses. Ten grams of pulverized sample was combined with 10 mL of 7.2% perchloric acid and 20 mL of cold distilled water. After blending, the sample mixture was filtered and the TBARS reagent added. The absorbance was read at 529.5 nm, and a standard equation was then used to determine the TBARS concentration.

Fatty Acid Profiles. Subcutaneous fat samples were taken from either the top sirloin butt or the shoulder clod of each carcass, frozen in liquid nitrogen and pulverized. A Shimadzu model GC-9AM gas chromatograph was used to analyze the samples. Proportions of 14:0, 16:0, 16:1, 18:0, 18:1, and 18:2 fatty acids were determined.

Statistical Analyses. The Correlation procedure of SAS was used to generate correlation coefficients and probablility values. Probablility values of less than 0.05 were considered significant.

Results and Discussion

Myoglobin and Hemoglobin. Myoglobin and hemoglobin levels were analyzed for correlations with sensory panel livery flavor intensity scores. Significant correlations involving myoglobin resulted for four treatment combinations (Table 1), although these correlations were somewhat low. However, myoglobin content of muscles may contribute to the intensity and incidence of livery flavor.

No correlations of hemoglobin with livery flavor were significant. No other published research has attempted to correlate pigment concentration with livery flavor.

Gas Chromatography/Mass Spectrometry. Table 2 contains a list of volatile compounds that frequently differed in concentration between samples that had livery flavor as rated by the trained flavor-profile sensory panel and those randomly selected that did not have livery flavor. Thirteen volatile compounds were in greater amounts in the samples that had livery flavor, whereas three were greater in selected samples that did not have a livery flavor. Other compounds (85) found in both livery and non-livery samples, were in proportions not different between sample types

Lipid Oxidation. As expected, longer aging time resulted in increased TBARS values ($P<0.05$; Table 3). However, the TBARS values for 35-day aged steaks were much lower than a level that could influence oxidative flavor. No significant ($P>0.05$) correlation existed between the sensory panel livery flavor attribute and TBARS values within any treatment combinations, possibly a result of very little variation in the low TBARS values.

Fatty Acid Profiles. Although some statistically significant correlation coefficients were identified (Table 4), no moderate or strong relationships were found. Even so, some unsaturated fatty acids may contribute to livery flavor in certain muscles.

Livery flavor was not strongly related to lipid oxidation. Muscle myoglobin concentration in some muscles may influence livery flavor. Thirteen different volatile compounds occur in greater amounts in samples with livery flavor. The undesirable livery off-flavor attribute is complex and likely affected by a number of muscle components, with no major contributor yet identified.

Table 1. Significant Correlation Coefficients ($P<0.05$) of the Livery Flavor Attribute with Myoglobin Concentration within Muscle, Aging Time, and Marbling

Muscle	Aging Time	Marbling	Pigment	Correlation Coefficient
Top sirloin	7 days	Slight	Myoglobin	0.34
Top blade	7 days	Slight	Myoglobin	0.28
Top blade	35 days	Slight	Myoglobin	0.35
Tenderloin	7 days	Small	Myoglobin	0.39

Table 2. Volatile Compounds Found to be Higher in Livery Samples versus Non-Livery Samples

Compound Name
(Compounds with higher concentrations in livery samples)
Hexanal
Butane, 1-(ethenylthio)
dl-Limonene
2-Octenal
Nonanal
2-Nonenal, (E)-
2-Decenal-[E]-
2,4-Decadienal, (E,E)-
2,4-Decadienal
2-Undecenal
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-
Pentan-1,3-Dioldiisobutyrate, 2,2,4-tryme
Tetradecanal
(Compounds with higher concentrations in non-livery samples)
Trans-2-Undecen-1-ol; or Dodecanol
Octadecanal or Hexadecanal
Octadecanal

Table 3. Aging Time Effects for 2-Thiobarbituric Acid (TBARS) Values from Steaks

Aging Time	TBARS Value (ppm)
7 days	0.06[b]
35 days	0.13[a]

[ab]Means within a column having different superscript letters differ (P<0.05).

Table 4. Significant Correlation Coefficients (P<0.05) of Livery Flavor Attribute with Fatty Acid Concentrations in Selected Muscle x Aging Time Combinations

Muscle	Aging Time	Fatty Acid[a]	Correlation Coefficient
Gluteus medius	7 days	18:1n9t	-0.20
Gluteus medius	14 days	18:1n9t	-0.20
Gluteus medius	14 days	18:1n9t	0.21
Gluteus medius	35 days	16:1	-0.19
Gluteus medius	35 days	17:1	-0.19
Psoas major	35 days	18:2n6c	-0.19

[a]n9t = carbon number 9 trans; n6c = carbon number 6 cis.