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# Effects of total iron, myoglobin, hemoglobin, and lipid oxidation of uncooked muscles on livery flavor development and volatiles of cooked beef steaks ☆

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**Abstract**

*Infraspinatus* (IN), *gluteus medius* (GM), and *psoas major* (PM) steaks were obtained from A- and B-maturity carcasses with either high ($\geqslant 6.0$) or normal ($\leqslant 5.7$) pH, and either Slight or Small marbling. Steaks were vacuum aged either 7, 14, 21, or 35 d postmortem, and were broiled and served to a highly trained, flavor-profile sensory panel. Steaks with livery flavor were analyzed by gas chromatography/mass spectrometry for flavor compounds. Steaks aged 7 or 35 d postmortem were analyzed for myoglobin (Mb) and hemoglobin (Hb) concentrations and for total iron (Fe) (35 d steaks only). The IN had greater Fe ($P < 0.05$) than did the GM or PM. Livery flavor increased ($P < 0.05$) and beef flavor identification decreased ($P < 0.05$) in the GM as Fe increased. The PM had the lowest ($P < 0.05$) Mb/Fe ratios and highest ($P < 0.05$) Hb/Fe ratios. Several statistically significant, but relatively low correlations between 16-, 17-, and 18-carbon chain fatty acids and livery flavor resulted. Thirteen volatile compounds had higher concentrations in steaks with livery flavor than in those without livery flavor. Livery flavor development is a complex trait that can be affected by concentrations of total Fe, Mb, and fatty acids, but the relationships are relatively low.


*Keywords:* Beef; Livery flavor; Oxidation; Pigment concentration; Lipids; Volatiles

## 1. Introduction

Livery flavor has been identified as a common off-flavor associated with beef (Campo, Sanudo, Panea, Alberti, & Santolaria, 1999; Eilers et al., 1994; Morris, Theis, Miller, Acuff, & Savell, 1997). Camfield, Brown, Lewis, Rakes, and Johnson (1997) found that vaccenic acid was negatively related to the livery, sour, and metallic attributes. Larick, Turner, Koch, and Crouse (1989) found that an increase in the lysophosphatidylcholine content was related to increased intensity for the ammonia, gamey, and livery attributes in beef and bison steaks. They also found an increase in the intensity of the ammonia, bitter, gamey, and liverish flavor attributes as the phosphatidylethanolamine content increased. Previous research has indicated that phosphatidylethanolamine also influences the incidence of warmed-over flavor attributes (Igene & Pearson, 1979).

Little research has been conducted on the volatiles responsible for livery flavor. Mussinan and Walradt (1974) stated that pork liver had a stronger odor than beef

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liver, and they found that the majority of compounds associated with cooked pork liver were pyrazines. Lorenz et al. (1983) conducted simultaneous steam distillation and solvent extraction of sheep liver and found a total of 108 compounds. Many of these compounds were categorized as aldehydes, esters, and hydrocarbons, but they also identified 15 thiazoles, 9 pyrazines, and 5 pyridines. Werkhoff et al. (1996) identified approximately 120 sulfur compounds from cooked beef liver by mass spectrometry. The sulfur compounds associated with cooked beef liver included thiols, sulfides, disulfides, thiazoles, and sulfur-substituted furans. Gorraiz, Beriain, Chasco, and Insausti (2002) found that livery flavor intensity of cooked beef was positively correlated with ethanal content, and they found that 2-propanone was positively correlated with livery flavor and odor intensity as well as with bloody flavor intensity.

No research has been conducted to determine if total iron, myoglobin, or hemoglobin concentrations are related to the development of livery flavor, but it is possible that they influence livery flavor. Because very little research has been conducted on the effects of fatty acids or the volatiles responsible for livery flavor, our objectives in this study were to determine if total iron, pigment concentration, lipid oxidation, and fatty acid composition were related to livery flavor development, and to identify the volatile compounds that were related to livery flavor development in different beef muscles of differing maturity, pH, marbling, and postmortem age.

## 2. Materials and methods

### 2.1. Subprimal and muscle selection

Three muscles from three different beef subprimals identified by NAMP (1997) nomenclature were removed from beef chuck, shoulder clods (NAMP 114, 1997); loin, top sirloin butts (NAMP 184); and loin, full tenderloins (NAMP 189). These subprimals were obtained from two commercial beef slaughter and processing facilities at six different sampling times. The *infraspinatus* (IN), *gluteus medius* (GM), and *psoas major* (PM) muscles were excised from each of the respective subprimals at 7 d postmortem. Carcasses were selected to fit into two groups: (1) A-maturity bone and (2) B-maturity bone. These groups were further selected to be of two pH subgroups: (1) those having a pH of 5.7 or less and (2) those having a pH of 6.0 or greater as measured by using a Sentron Argus pH meter with a Lance FET pH probe (Sentron, Northbridge, MA). In selecting carcasses for our research, *longissimus thoracis* muscle pH was measured at 24–48 h postmortem. Carcasses also were 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$^{00}$. Due to the low incidence of B-maturity × high pH carcasses, we were unable to select an equal number of these carcasses. Individual muscles were removed from the subprimals at 7 d, and steaks 2.54-cm thick were cut, randomly assigned to an aging treatment (7, 14, 21, or 35 d), and vacuum packaged. The IN was cut into steaks perpendicular to the muscle bundles. The GM was cut into steaks anterior to posterior, and the PM was cut into steak pairs (1 + 8, 2 + 7, 3 + 6, and 4 + 5) because of their smaller size and the pairs were randomized to aging treatments. The steaks assigned to the 7-d aging treatment were placed in a freezer at $-52 \pm 1$ °C until just prior to evaluations by a trained flavor-profile sensory panel. The remaining steaks were aged at 2–4 °C either 14, 21, or 35 d postmortem. All steaks were then frozen and stored at $-52 \pm 1$ °C until evaluation.

### 2.2. Total iron content

Total iron (Fe) content of uncooked steaks (35 d) was measured according to AOAC method 968.08 (1995). A 3.0- to 3.5-g sample pulverized in liquid nitrogen was weighed into a ceramic crucible. Samples were ashed in a muffle furnace (model 85A, Neytech, Bloomfield, CT) at 600 °C for 4 h. After samples were cooled to 20 °C, they were digested with 2.5 N of HCl for 50 min. Digested samples were diluted to 50 ml with distilled-deionized water and mixed. An atomic absorption spectrophotometer (AAnalyst 100, Perkin Elmer, Norwalk, CT) was used to measure sample absorbance at 248.3 nm. Comparisons were made to a standard curve using 0, 1, 2, 5, and 10 ppm of iron.

### 2.3. HPLC quantification of myoglobin and hemoglobin

Muscle samples were pulverized by using liquid nitrogen. A 15-g portion was placed in a stomacher bag, and 25 ml of a sodium phosphate buffer was added to the bag. The bag was stomached for 1 min, and the extract was poured into a round-bottom centrifuge tube and centrifuged at 21,500*g* for 30 min in a Beckman centrifuge (J2-J21, JA17 rotor, Beckman Coulter, Fullerton, CA) at 2 °C. The supernatant was filtered through Whatman No. 1 filter paper, and a 2-ml aliquot was pipetted into an amber-colored auto sampler vial containing 0.1 g of sodium dithionite and then shaken. The reaction was allowed to proceed a minimum of 10 min before the solution was passed through a 0.45-μm filter. The sample was injected into the HPLC (Hewlett–Packard Series II, 1090A HPLC; Agilent Technologies, Palo Alto, CA) within 5 min of filtering to prevent hemoglobin (Hb) from returning to an oxidized state. A TSKgel Phenyl-5PW 7.5 cm × 7.5 mm column with a 10-μm particle size (Tosoh Biosep, Montgomeryville, PA) was used for all analyses. The system was set for a linear gradient (100% A to B) over 15 min. A 50-μl buffered (Oellingrath, Iversen, & Skrede, 1990) volume of the filtered sample was injected into a 100-μl loop. The run time was set for 17 min. The HPLC instrument and the ultraviolet spectrum were monitored at 420 nm. Horse skeletal-muscle myoglobin (Mb), bovine