FACTORS INFLUENCING OFF-FLAVOR IN BEEF

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FACTORS INFLUENCING OFF-FLAVOR IN BEEF

by

Jennie Marie James Hodgen

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Animal Science

Under the Supervision of Professor Chris R. Calkins

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Projects were conducted to increase knowledge of liver-like off-flavor origins in muscles from the beef chuck and round. Effects of cooking rate and holding time on off-flavor of various steaks from ten carcasses were determined. Off-flavor from these muscles was lowest when the steaks were cooked slowly (on a 149°C grill versus a 249°C grill) and when held for one h prior to evaluation. The *M. infraspinatus* had the least off-flavor, and the *M. vastus intermedius* had the most intense off-flavor. These data suggest a carcass with one off-flavored muscle is likely to have other off-flavored muscles in the chuck and round. It appears the off-flavors are aromatic volatiles as off-flavored samples could be differentiated during cooking. Investigations to identify compounds causing beef off-flavors were undertaken. A protocol was developed to capture volatile compounds from raw, pulverized meat samples in a polymer column and elute the volatiles with ethyl ether for injection into a gas chromatograph (GC). Differences in peak height/area could be seen between samples identified as normal and liver-like in flavor. Compound identification using the ether sample was implausible with GC-mass spectrometry (GC-MS) so samples were run in a purge and trap GC-MS system (PT). Compound differences in normal
and liver-like samples were those associated with lipid oxidation; β-pinene, 1-octen-3-ol, and 2,4-decadienal were higher in concentration in liver-like off-flavored samples in four muscles tested, as well as in raw liver. Solid phase microextraction (SPME) with GC-MS validation identified the presence of similar compounds identified with PT in addition to differences in lower molecular weight compounds in liver-like samples not detectable in the previous study. Lipid oxidation compounds are at least partially responsible for liver-like off-flavor, and different muscles have their own unique volatile profile. Twenty-eight compounds were found in all four raw normal flavored muscles. *M. triceps brachii* had the fewest compounds, while *M. rectus femoris* had the most compounds with ten unique from those in other muscles.

**Keywords:** Beef, Off-flavor, Volatile Compound Identification
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Every 60 seconds you spend upset is a minute of happiness you can never get back.
TABLE OF CONTENTS

ABSTRACT ..............................................................................i

ACKNOWLEDGEMENTS........................................ iii

TABLE OF CONTENTS......................................................v

INTRODUCTION ..............................................................1

REVIEW OF LITERATURE

1. Flavor
   a. General Introduction ...........................................3
   b. Olfactory System................................................4
   c. Chemistry of Taste..............................................9
      i. Salty...............................................................10
      ii. Sour.............................................................10
      iii. Sweet..........................................................11
      iv. Bitter ..........................................................12
      v. Umami ..........................................................13
      vi. Taste receptor summary...............................14
   d. Mastication and Swallowing...............................14
   e. References ......................................................17
   f. Figures
      i. Figure 1. Frontal section of rabbit and human nasal cavity ....22
      ii. Figure 2. Primary olfactory pathway..23
      iii. Figure 3. Olfactory mucous membrane ..................24
      iv. Figure 4. Known and proposed pathways of olfactory transduction .................................25
      v. Figure 5. Odor introduction to nasal cavity and stimuli pathway to the brain ........26
      vi. Figure 6. Transformation of odorant receptor inputs in the nervous system........27
      vii. Figure 7. Ortho- and retronasal routes of aroma perception .......................28
      viii. Figure 8. Difference in sniffing and breathing in .........................29
ix...Figure 9. The taste bud ......................30
x. Figure 10. Functional anatomy of the
tongue .................................31
xi. Figure 11. The velum and tongue........32
xii. Figure 12. Perceptual interactions
evoked during ingestion ....33
g. Table
i. Table 1. Physiological factors in the
mouth during eating ..........34

2. Beef Flavor
a. Introduction........................................35
b. Beef Species Flavor............................36
c. Live Animal Factors..............................37
   i. Heritability ................................37
   ii. Animal gender .........................37
   iii. Animal age ..........................39
   iv. Diet ..................................41
d. Aging ........................................49
e. Muscles ........................................52
f. Maillard reaction .................................55
   i. Amadori rearrangement ...............55
   ii. Strecker degradation .................56
   iii. Schiff base .........................56
   iv. Melanoids ...........................56
   v. Product compounds .................56
   vi. pH ..................................57
g. Fat ...........................................57
   i. Fat content ..........................58
   ii. Triacylglycerides and phospholipids ..58
   iii. Fatty acids .........................59
h. Other ........................................61
   i. Thiamin ................................61
   ii. Sulfur ................................62
   iii. pH ..................................63
   iv. Degree of doneness ..................63
   v. Other contributing factors ..........65
i. Compounds ................................65
j. References ..................................68
k. Figures
   i. Figure 1. Compounds that possess
      meaty odor .........................80
   ii. Figure 2. Diagram of Maillard
      reaction in foods .................81
iii. Figure 3. Amadori rearrangement end products ..................................82
iv. Figure 4. Meat thiols, sulfides, and disulfides that contribute to meaty aroma ..........83

l. Tables
   i. Table 1. Flavor intensity ranking of muscles ........................................84
   ii. Table 2. Off-flavor intensity ranking of muscles ..................................85

3. Methods to Isolate Volatile Compounds
   a. Introduction ......................................................................................86
   b. Static Headspace .............................................................................86
   c. Solid Phase Micro Extraction .........................................................87
   d. Purge and Trap GC/MS ..................................................................90
   e. Other .................................................................................................94
   f. Comparison Between Methods ..........................................................94
   g. References .........................................................................................95
      i. Figure 1. Static Headspace ..............................................................97
      ii. Figure 2. Direct SPME versus headspace SPME .........................98
      iii. Figure 3. Diagram of SPME MALDI ..........................................99
      iv. Figure 4. Diagram of SPME/Nanospray ....................................100
      v. Figure 5. Purge and trap modes of action ..................................101

MATERIALS AND METHODS

1. The influence of cooking rate and holding time on beef chuck and round flavor ........................................102
   a. Sample collection ........................................................................102
   b. Panelist training ........................................................................103
   c. Sensory analysis ........................................................................105
   d. Chemical analysis ........................................................................107
   e. Statistical analysis ........................................................................109

2. Protocol for determining volatile compound differences between liver-like and normal beef samples using gas chromatography ........................................109
   a. Sample preparation ......................................................................110
   b. Collection of volatile compounds ..............................................110
   c. Gas chromatography ...................................................................111
   d. Mass spectrometry ......................................................................112
3. Identification of volatile compounds in beef chuck and round muscles ..................................................113
   a. Justification for the Purge and Trap method .113
   b. Purge and Trap Mass Spectrometry ...............113

4. Uncooked beef muscles with liver-like flavor are similar in volatile compounds to raw beef liver .......115

5. Validation of the purge and trap mass spectrometer results with SPME using M. triceps brachii and verification of compounds with M. rectus femoris ....116
   a. SPME validation ........................................116
   b. Verification of compounds found in the M. rectus femoris with Purge and Trap mass spectrometry ........................................117

a. References........................................................................119

MANUSCRIPTS

1. The influence of cooking rate and holding time on beef chuck and round flavor
   a. Title .................................................................120
   b. Abstract ..........................................................121
   c. Introduction ......................................................122
   d. Materials and Methods ........................................124
   e. Results and Discussion ........................................128
   f. Implications ......................................................135
   g. References ........................................................136
   h. Tables
      i. Table 1. Chemical analysis of seven muscles ..............138
      ii. Table 2. Cooking and holding loss of M. teres major, M. vastus intermedius, and M. vastus medialis ......................139
      iii. Table 3. Cooking and holding loss of M.infraspinatus, M. triceps brachii, M. rectus femoris, and M. vastus lateralis ..........140
      iv. Table 4. Cooking times for muscles cooked FAST and SLOW .......141
      v. Table 5. Sensory evaluation of M. teres major, M. vastus intermedius, and M. vastus medialis ..........142
vi. Table 6. Sensory tenderness and connective tissue scores from
M. infraspinatus, M. triceps brachii, M. rectus femoris, and
M. vastus lateralis.....................143
vii. Table 7. Sensory juiciness scores from
M. infraspinatus, M. triceps brachii, M. rectus femoris, and
M. vastus lateralis.....................144
viii. Table 8. Sensory off-flavor intensity
Scores from M. infraspinatus,
M. triceps brachii, M. rectus femoris, and M. vastus lateralis .......................145
ix. Table 9. Percentage of panelists denoting specific off-flavors for
M. teres major, M. vastus intermedius, and M. vastus medialis .......................146
x. Table 10. Percentage of panelists denoting specific off-flavors for
M. infraspinatus, M. triceps brachii, M. rectus femoris, and
M. vastus lateralis.....................147

2. Protocol for determining volatile compound differences between liver-like and normal beef samples using gas chromatography
a. Title...............................................................148
b. Abstract ..........................................................149
c. Introduction......................................................150
d. Materials and Methods........................................151
e. Results and Discussion.......................................154
f. Conclusion.........................................................155
g. References.........................................................157
h. Tables
   i. Table 1. Times of undesirable smells coming out of gas dispersion container using different nitrogen gas flow rates for samples rated as off-flavored.......................161
   ii. Table 2. Retention times and areas for off-flavored and normal M. rectus femoris samples ......................162
i. Figures
   i. Figure 1. Gas dispersion container.............158
ii. Figure 2. Gas chromatograms from off-flavored and normal samples. 159

iii. Figure 3. Chromatograms from GC-MS from off-flavored and normal

M. rectus femoris ...................... 160

3. Identification of volatile compounds in beef chuck and round muscles
   a. Title ................................................................. 163
   b. Abstract .......................................................... 164
   c. Introduction ..................................................... 165
   d. Materials and Methods ........................................ 166
   e. Results and Discussion ........................................ 168
   f. Conclusion ........................................................ 173
   g. References ...................................................... 175
   h. Tables
      i. Table 1. Compound concentration differences between liver-like and normal-flavored beef muscles ............... 179
      ii. Table 2. Classification of volatile compounds identified by mass spectrometry .................................... 180
      iii. Table 3. Compound concentration differences for 0-11 min between normal-flavored beef muscles ...................... 181
      iv. Table 4. Compound concentration differences for 11-22 min between normal-flavored beef muscles ...................... 182

i. Figures
   i. Figure 1. Chromatogram from off-flavored M. triceps brachii ............... 183
   ii. Figure 2. Chromatogram from normal-flavored M. triceps brachii ... 184

4. Uncooked beef muscles with liver-like flavor are similar in volatile compounds to raw beef liver
   a. Title ................................................................. 185
   b. Abstract .......................................................... 186
   c. Introduction ..................................................... 187
   d. Materials and Methods ........................................ 187
   e. Results and Discussion ........................................ 188
   f. References ...................................................... 191
g. Tables
   i. Table 1. Volatile compounds found in Raw beef liver .......................... 192
   ii. Table 2. Volatile compounds in both liver and in higher concentration in liver-like samples compared with normal samples .......... 193

5. Validation of the purge and trap mass spectrometer results with SPME using *M. triceps brachii* and verification of compounds with *M. rectus femoris*
   a. Title ........................................................................................................ 194
   b. Abstract .................................................................................................. 195
   c. Introduction ............................................................................................ 196
   d. Materials and Methods ........................................................................ 197
   e. Results and Discussion ......................................................................... 199
   f. Conclusion ............................................................................................. 201
   g. References ............................................................................................. 203
   h. Tables
      i. Table 1. Volatiles identified with SPME compared to compounds identified from purge and trap method for the *M. triceps brachii* ...................... 204

RECOMMENDATIONS FOR FUTURE WORK ... 205

APPENDICES

1. Appendix 1. Example of Taste Panel Ballot ............................................ 217
2. Appendix 2. Compounds Characteristics .............................................. 218
Introduction

Flavor is one of the most important attributes of beef palatability. This attribute can ultimately affect the consumer’s acceptance of a beef product and purchasing habits toward buying beef.

With the increased utilization of muscles from the chuck and the round for steaks instead of roasts or ground beef, the value of the chuck and round has seen an increase in value since 1998. Most of the increased usage was initially for foodservice providing an affordable, yet high quality product in banquet type settings. Anecdotally, managers of foodservice establishments indicated they were receiving increased numbers of complaints about off-flavored beef samples described as tasting like liver.

In foodservice, meat entrees are typically cooked and then held in a warming oven as the other items are added to the plate and more entrees can be prepared. With most of the off-flavor complaints at the time stemming from foodservice establishments, it was hypothesized that the cooking rate and holding time might influence the production of off-flavors in the steaks from chuck and round muscles.

In the initial study investigating cooking rate and holding time effects on flavor, several key observations were made that led to the development of several subsequent studies, including the remaining four manuscripts in this dissertation. A primary observation was that off-flavors are aromatic volatiles since off-flavored samples could be differentiated during cooking.

Hypothetically, by capturing the volatiles and identifying them, one could work backwards to find out what environmental or genetic factors are responsible for a specific animal having off-flavored meat. Therefore, the objectives of this research were to 1)
develop a method to identify differences in compounds between normal and off-flavored samples, 2) use the purge and trap gas chromatography mass spectrometer method to identify the compounds that were different, 3) determine if the compounds in the liver-like off-flavored samples were related to the flavor compounds in liver, and 4) validate and verify the results were reproducible and accurate. The end goal was to be able to offer hypotheses as to the possible origins of the compounds that were creating the liver-like off-flavor.
Review of Literature

Part 1. Flavor

General Introduction

The term flavor comes from the Middle English word flavour which is a modification of the Anglo-French flaure/flour that comes from the Latin word flator, an alteration of the Latin word flatus which means breath or act of blowing (Merriam-Webster, 2006). The noun form of the word flavor has several definitions: “odor, fragrance”, “the quality of something that affects the sense of taste”, “the blend of taste and smell sensations evoked by a substance in the mouth characteristic or predominant quality”, “a distinctive appealing or enlivening quality”, “a property that distinguishes different types of elementary particles (as quarks or neutrinos)”, and/or “any of the different types of particles that are distinguished by flavor”; the verb form of flavor means “to give or add flavor to” (Merriam-Webster, 2006).

In general, most food scientists refer to flavor as the combination of taste and aroma. A common experiment to illustrate how important aroma is to the perception of flavor is to have an individual hold his/her nose, close his/her eyes, and try to guess whether he/she is eating an apple or a potato. Drs. Susan Schiffman, Duke University psychiatry professor of taste and smell disorders, and Alan Hirsch, founder and neurological director of the Smell and Taste Treatment and Research Foundation in Chicago, have stated that aroma/smell makes up 80% (Chicago Tribune, 1990) or 90% (Melbourne, 2003) of flavor, respectively. Taste, even without swallowing, is also very important to flavor as illustrated by Mattes (2001) where his studies revealed serum triacylglyceride levels increased when fatty food was masticated for 10 sec and
expectorated without swallowing. Therefore, to perceive flavor, the volatiles in the food are identified through the nose (smell) and the non-volatiles are identified with the mouth (taste).

Off-flavor is a perception. An individual’s perception of acceptability of food flavor is affected by numerous factors such as age, health, food availability, environment, and culture. From the definition of flavor one can see that off-flavor is encompassed in the term flavor because a certain amount of a specific compound(s) is affecting the sense of taste and odor. However, in order for individuals to describe a flavor that is not perceived as normal, the term off-flavor has been utilized.

To gain a better understanding of factors in a food system that may affect flavor, a basic knowledge of the biology of the olfactory system and the chemistry of taste is needed.

**Olfactory System**

The olfactory system is interesting and complex because the cells that make up this system must be able to identify one or more molecules and then derive a meaningful response. The cells that make up the olfactory system are different from cells that make up the sensory taste cells in that stimuli from the dendrite to an axon carries a message directly to the central nervous system. However, taste and smell cells are related in that they both are exteroceptors because they respond to chemical stimuli originating from outside the body (Farbman, 1992).

Humans are considered microsmatic (Figure 1), meaning they are mammals with relatively poor sense of smell because of minimal surface area of the turbinals (Farbman 1991). Figure 2 demonstrates how the stimuli can come into contact with the dendrite
appendages, travel down around the cell body through a single unmyelinated axon, and cross the synapse to the central nervous system (CNS).

The epithelium/connective tissue layer and the lamina propria make up the mucous membrane in the roof of the nose with a thin membrane separating the two layers (Figure 3). The epithelium contains the sensory cell, the sustentacular cell, and the basal cell. The dendrite extensions near the surface are cilia except in the vomeronasal which has microvillus dendrite extensions. Menco (1980) found the cilia extensions on the dendrites increase the surface area 25 to 40 times which increases the responsiveness of the olfactory system since this is the first part of the structure that comes into contact with odors in the nasal cavity. The cell body is usually in the middle to lower third of the epithelium and contains a nucleus, nucleolus, chromatin, ribosomes, endoplasmic reticulum, golgi apparatus, and lysosomes (Farbman, 1992). The role of the sustentacular (supporting) cell is thought to regulate passage of substances between the surface and the connective tissue (Getchell, Margolis, & Getchell, 1984) as well as detoxification (Reed, Lock, & De Matteis, 1986). The basal cells are assumed to produce new neuronal cells (Graziadei & Monti Graziadei, 1979).

The septal olfactory organ is in a small part of the epithelium that can detect a broad range of odors and is more sensitive than the main nasal cavity (Marshall & Maruniak, 1986). The axons from the septal organ form fascicles separate from the vomeronasal and main fascicles to go to the olfactory bulb (Farbman, 1992).

The lamina propria, usually at least twice as thick as the epithelium, contains blood vessels that allow blood flow to the mucosa as well as connective tissue for support, Bowman’s glands, and olfactory, trigeminal (detection of heat, cold, and pain),
vomeronasal, and terminal nerve bundles. The Bowman’s glands provide most of the mucus on the epithelial surface to protect against drying out and invading agents. Therefore the odorants must diffuse through the mucus in the nasal cavity to reach the dendrites (Farbman, 1992).

The initial step in detection of odors is the interaction between the stimulus and the olfactory cell. Odorant-binding proteins, located near the olfactory dendrites and surrounded by mucus, help solubilize odor molecules and transport odorants to receptor proteins on dendrites (Pevsner, Sklar, & Snyder, 1986; Pevsner, Hou, Snowman, & Snyder, 1990). Axel and Buck (1991) won The Nobel Prize in Physiology or Medicine in 2004 for discovering the gene family that encoded for these odor receptors. Further research found the odor molecules can then interact through G-protein-coupled receptors with the olfactory receptor neurons in the main olfactory organ to produce secondary messengers by two pathways (Figure 4): cAMP and InsP$_3$ (Ache & Zhainazarov, 1995).

The first synaptic junction in the olfactory system is the olfactory bulb, a paired organ on each side of the bottom midline of the forebrain. The axon bundles extend through the nasal cavity roof and end at the olfactory bulb (Figure 5). The olfactory bulb has seven layers: olfactory nerve, glomerular layer, external plexiform layer (EPL), mitral cell body layer, internal plexiform layer, granule cell layer, and subependymal layer (Frabman, 1992). The axon bundles are woven together at the nerve, and the glomerular layer is the synaptic space between the axons from the nasal cavity to the olfactory bulb. The EPL is where the olfactory process begins as the impulse arrives from the synaptic space while the mitral cells relay information to the olfactory cortex. The granule layer also helps with processing the signals received from the axons (Farbman, 1992). Because
of the ‘one way in, one way out’ structure of the olfactory bulb, it is assumed that its main function is to filter impulses as well as return responses which allows this organ to enhance discrimination between odors, enhance sensitivity, and filter out background aromas (Shepherd, 2003).

Olfactory cortex, which is made up of several systems in the brain, receives information (action potentials) from the olfactory bulb that allows an individual to become conscious of or identify odors. Zou, Horowitz, Montmayeur, Snapper, & Buck (2001) illustrated with gene knockout experiments in mice that neurons receive signals and pass the information down the olfactory nerve through the olfactory bulb to specific locations within multiple parts of the olfactory cortex (Figure 6). Therefore, it was suggested the inputs from the same receptors are being processed at the same time in different areas to allow better detection and sensitivity to smells. More recent work (Anderson et al., 2003) demonstrated the amygdala activation is spurred by intensity of an odor, while valence (pleasantness/unpleasantness) is associated with the orbitofrontal cortex, which is a secondary taste cortex because it receives signals from the amygdala or piriform cortex (Rolls, 1999).

Orthonasal and retronasal are the two methods by which odor particulates can reach the epithelium (Figure 7). Orthonasal can be both passive (normal inhalation) and active (Figure 8), with the latter obtaining stronger intensities with sniffing (Laing, 1983). While the concept was introduced by Rozin (1982) several studies have now demonstrated that orthonasal and retronasal perceptions are not the same (Voirol & Daget, 1986; Pierce & Halpern, 1996; Helimann & Hummel, 2004; Small, Gerber, Mak, & Hummel, 2005; Pfaar, Landis, Frasnelli, Huttenbrink, & Hummel, 2006). Results from
these studies vary as to which type can perceive lower thresholds. The results seem to depend on the food or odor type and the other variables (background odors, multiple samples, manner in which the orthonasal/retronasal sense was bypassed in the studies, etc).

**Metallic**

Metallic is generally not considered a taste even though it has extremely low volatility. Hettinger, Myers, & Frank (1990) and Lawless et al. (2004) found with ferrous sulfate, the metallic flavor was retronasal, not gustatory, but Lawless et al. (2004) did report that one panelist reported a ‘metallic feeling on the tongue’. It was hypothesized the metallic compounds were perceived with ferrous sulfate because of catalysis of lipid oxidation in the mouth (Lawless et al., 2004). Buettner & Schieberle (1999) found several compounds related to aromatic end products of oxidation of linoleic acid that were perceived to be metallic or cause unpleasant flavors in gas chromatography-olfactory. In the FeSO₄ model system, the retronasal threshold was 0.015 ppb (Buettner & Schieberle, 1999); however, the mechanisms of the metallic sensation are unknown at this time (Lawless et al., 2004). Panelists often refer to metallic as a taste rather than an aroma, as it has clearly been demonstrated to be (Hettinger et al., 1990). To clarify this confusion for panelists, sensory evaluations are using metallic mouthfeel, aftertaste, and/or aroma as descriptors (Miller, Rockwell, Lunt, & Carstens, 1996; Camfield, Brown Jr, Lewis, Rakes, & Johnson, 1997; Mandell, Buchanan-Smith, & Campbell, 1998).

Research is being conducted on improvements to techniques to evaluate aromas by machines and by humans as well as studying olfaction on a cellular level. Besides benefiting the food industry to help create more complete flavors, this continued effort
also should contribute to improving the quality of life for individuals that have or will develop anosmic conditions.

**Chemistry of Taste**

For years the common theory was humans possessed four basic taste buds: sweet, sour, bitter, and salty. Recent research has demonstrated the ability to taste is much more complicated than previously thought. In fact umami, a Japanese phrase loosely translated as savory, deliciousness, or meaty that is derived from the sensation of glutamate, is starting to be considered a fifth taste (Lindemann, 2001).

Taste buds, located within papillae on the tongue and soft palate, house between 50-100 taste cells per taste bud. The taste cells extend microvilli to the taste pore, the opening in the taste bud to the surface of the tongue (Figure 9). The largest group of papillae in the mouth does not contain taste buds but rather are involved with mouthfeel. The papillae that house the taste buds are the fungiform on the front of the tongue, the circumvallate distributed in a V shape at the back of the tongue, and the foliate situated on the sides of the rear of the tongue. While the distribution may be similar to traditional tongue maps, no specific taste is isolated in one area, but is instead located in three of the four papillae regions (Figure 10).

To identify a specific taste, a tastant (chemical from food) comes in contact with the microvilli of the taste cell in the taste pore. Depending on the taste, the tastant can react in two ways: interact with the proteins on the cell surface or with the ion channels. Both interactions cause a change in the electrical charge that causes signals to be sent to the brain. Like other cells, taste cells maintain a negative charge internally so when
tastants alter the electrical state, neurotransmitters send a message by depolarization to the brain.

Like the receptors in the olfactory system, the receptors for taste can also detect multiple chemical types although most are more sensitive to one taste than another and several receptors have been isolated for specific tastes. However, salt and sour have traditionally been assumed to go through ions channels (Kinnamon & Margolskee, 1996) whereas, sweet and bitter bind to receptors that open and close the cell’s ion channels. McLaughlin, McKinnon, and Margolskee (1992) identified gustducin, a G-protein, which is critical for perceiving sweet and bitter. Recent studies suggest that all tastants bind to receptors and are innervated by fibers that send the information to the central nervous system or cortex through a synapse in the brain stem and thalamus (Zhao et al., 2003). A brief description of each of the basic tastes follows.

Salty

The entry of $\text{H}^+$ and $\text{Na}^+$ through the pores on the apical part of the cell is thought to control the salt taste function. No pathway components of this direct entry have been suggested, but depolarization may result because of the $\text{Na}^+$ entering into the amiloride-sensitive $\text{Na}^+$ channels.

Sour

The sour taste has served as a warning sign to mammals that food was spoiled or unripe. Sour was thought to be similar to salty because it appeared $\text{H}^+$ and $\text{Na}^+$ directly entered into the membrane channels in the apical surface of the cell to give the sensation of sour. When sour compounds entered, it had been suggested that $\text{H}^+$ blocked the $\text{K}^+$ channels or the sour compounds activated epithelial $\text{Na}^+$ channels, acid-sensing ion
channels, K⁺ channels, or H⁺-gated calcium channels (Kinnamon & Margolskee, 1996; DeSimone, Lyall, Heck, & Feldman, 2001; Lindemann, 2001). With the identification of selective receptor cells for sweet, umami, and bitter, it was suggested that salty and sour also had receptors to mediate the transduction of the taste. Huang et al. (2006) demonstrated PKD2L1, polycystic-kidney-disease-like ion channel, acted as a sour taste sensor in mammals. When gene knockout studies in mice were conducted, the animals could not detect sour/acid, but did respond to the other tastes (bitter, salty, sweet) suggesting PKD2L1 is specific for the sour taste.

**Sweet**

The preference for sweet (or food with sugar content) may have stemmed from the evolutionary need for calories. Fuller (1974) demonstrated discriminatory threshold differences could be seen with different levels of saccharin solutions which were not seen with bitter taste. The *Sac* loci were identified as the principle locus that allowed for determination of levels of sweetness (i.e. intensity). In terms of further research for identifying receptors for sweet, a challenge existed as humans have submillimolar to micromolar sensitivities to aspartame, monellin, and thaumatin while rodents cannot taste those substances (Danilova, Hellekant, Tinti, & Nofre, 1998). After Hoon, Adler, Lindemeier, Battey, Ryba, & Zuker (1999) discovered two novel families of G-protein receptors in the tongue and palate, T1R and T2Rs, more studies revealed that the T1R3 receptor was encoded by *Sac*. The T1R3 was found to be expressed in ~30% of cells in taste buds of the circumvallate, foliate, fungiform, and palate. Interestingly, T1R3 was coexpressed with T1R2 (another T1R receptor) in the circumvallate, foliate, and palate taste buds and T1R1 (another T1R receptor) in the fungiform and palate taste buds.
(Nelson, Hoon, Chandrashekar, Zhang, Ryba, & Zuker, 2001). The T1R2 and T1R3 function as a heteromeric receptor to respond to sucrose, fructose, saccharin, acesulfame-K, dulcin, and guanidinoacetic acid 1 and 2 (Li, Staszewski, Xu, Durick, Zoller, & Adler, 2002). The two receptors by themselves did not invoke a noticeable response, and when present together, no responses were detected for bitter or umami tastants (Nelson et al., 2001). In contrast, Zhao et al. (2003) found that T1R3 alone did respond to high concentrations (>300mM) of natural sugars, but not artificial sweeteners. Because of difficulty in assaying T1R1+T1R3, the authors hypothesized that all TR1 receptors encode for sweet receptors since they are coexpressed in distinct cell subsets, T1R2+T1R3 in the back of tongue and palate and T1R1+T1R3 in the front of the tongue (Nelson et al., 2001). It was later shown that T1R1+T1R3 was a receptor for L-amino acids, with some of the amino acids (alanine, glutamine, serine, threonine, and glycine) being perceived as sweet (Nelson et al., 2002). This research in conjunction with Adler, Hoon, Mueller, Chandrashekar, Ryba, & Zuker (2000) support the notion that sweet and bitter tastes are activated by completely different receptor cells.

**Bitter**

Even with taste chemistry in its infancy, bitter may be the most studied of the tastes. While sour detection has served a role in protection against eating spoiled food, bitter detection is very important in creating aversion to many naturally occurring poisonous substances. Several groups tried to identify receptors, but could never demonstrate specific expression in tissue and cells, validate the results, and support the results with genetics. However, the collaborating group at Howard Hughes Medical Institute and National Institute of Dental and Craniofacial Research identified the T2R
receptors that are exclusively produced with gustducin-expressing taste cells in ~15% of the papillae (except fungiform) on the tongue and palate epithelium (Adler et al., 2000). At least three T2Rs were identified as receptors for bitterness which supports McBurney & Gent (1979) statement that mammals can recognize a wide range of bitter substances even though they cannot distinguish between them. The results were confirmed in the mouse model and in vitro that bitterness perception was altered when the T2R receptors were altered or removed (Chandrashekar et al, 2000). Additional research demonstrated T2R5 has affinity for cycloheximide, T2R16 is a candidate receptor for β-glucopyranosides, hT2R14 is a candidate receptor for picrotoxinin, and hT2R44 and hT2R61/hT2R43 are receptors for denatonium, aristolochic acid, and 6-nitrosaccharin (Mueller, Hoon, Erlenbach, Chandrashekar, Zuker, & Ryba, 2005). Despite these compounds having different receptors, discrimination between the different bitter compounds was never shown, even though sweet, umami, sour, and salty were not affected. Additionally, this study found that bitter (tendency for adverse taste perception) and sweet (attractive taste perception) were completely separate functions. By altering the genes, the scientist could make mice averse to sweet and prefer bitter (Mueller et al., 2005).

Umami

There is still some uncertainty if there is a specific receptor for umami. Chaudhari, Landin, & Roper (2000) suggested mGluR4 as a candidate for the umami receptor while Li et al., (2002), Nelson et al. (2002) and Zhao et al. (2003) argued T1R1+T1R3 was an amino acid receptor, although uncertainty exists if T1R1+T1R3 is the principal or an additional umami receptor. Li et al. (2002) and Zhao et al. (2003)
supported the hypothesis that sweet and umami share a common receptor evolutionary origin.

*Taste Receptor Summary*

While the preceding discussion revealed each taste perception probably has specific receptors, there is still considerable speculation of the signaling pathways after activation by the receptors. One study demonstrated that bitter, sweet, and umami required a taste receptor protein channel, TRPM5 (taste receptor protein gene that encodes a functional channel), and phospholipase C (PLCβ2) (Zhang et al., 2003). The TRPM5 gene is activated by G-protein-coupled reactions, not by Ca^{2+}, InsP3, or internal stores of TRPM5 to mediate the taste channel. Therefore, a tastant activates a T1R or T2R receptor to stimulate G-proteins and turn on PLCβ2, which opens the transduction channel and allows the depolarization to occur (Zhang et al., 2003). This study also supported the hypothesis that salty and sour have distinct signaling pathways independent of TRPM5, unlike bitter, sweet, and umami.

*Mastication and Swallowing*

The previous sections have shown that flavor perception is dependent on both aroma and taste. However, the act of chewing and swallowing causes changes to the food which allows certain tastes or odors to be perceived. The first moment of olfaction of a food is orthonasal as the individual smells the aromas from the food, whether it is during cooking or as the food approaches the nose and mouth. Buettner, Beer, Hannig, Settles, & Schieberle (2002) demonstrated the main retronasal ‘aroma pulse’ is simultaneous with the swallow breath because the velum (soft palate) tongue border (Figure 11) is opened to allow odors up to the olfactory epithelium. In time intensity
studies, the maximum intensity of flavor is usually near the time of swallowing (Buettner et al., 2002). de Wijk, Engelen, & Prinz (2003) trained panelists to chew and swallow in five different manners to demonstrate flavor perception differences due to individual habits, and found people that eat with the most complex movements have the highest flavor intensity.

The initial opening of the velum-tongue border actually occurs as the mouth opens to accept the food which allows the individual a short retronasal odor impression. During mastication, the velum-tongue border opens and closes, although when food with a higher moisture content or extra food is in the mouth, the velum-tongue border opens fewer times so fewer aromas are perceived prior to swallowing. While it has not been demonstrated, mastication may cause a propulsion of aromatics into the air flow which would allow further retronasal perception, although its role olfactory perception would be minor (Buettner et al., 2002). Continuous retronasal aroma perception is not possible due to the physiological mechanisms necessary to allow odors to reach the olfactory epithelium. However, prolonged retronasal perception can persist as odorants from the food can absorb into the saliva (Buettner et al., 2002). Because food matrices are so complex, to gain a better understanding of the effect of mastication and swallowing on flavor perception, a knowledge of how the concentration of a specific odorant reacts (dissolved, absorbed, bound, entrapped, etc) in a specific food system and the oral cavity is needed (Buettner & Schieberle, 2000).

Miettinen (2004) summarized mastication, saliva, diffusion, binding, and temperature’s effect of food during eating (Table 1) as well as cross-modal and multi-modal effects of taste and olfaction. Rolls & Baylis (1994) introduced proof of the
interaction between taste and olfaction in neurobiological studies in which both taste and aroma stimulation was needed to activate specific neurons. Further research has shown it is not only taste and olfaction that lead to a flavor perception (Figure 12) but other attributes such as color (Delwiche, 2004; Johnson, 2006), texture/thickness (Cook, Linforth, & Taylor, 2003), and temperature (Delwiche, 2004) play a significant role in the development of the overall flavor perception of food.
References


Figure 1. On the left is a diagrammatic representation of a frontal section through a rabbit nasal cavity, showing elaborate scrolling of the ecoturbinals and endoturbinals on the lateral aspect of the nasal cavity. On the right is a diagram of a frontal section through an adult human nasal cavity, showing superior (S), middle (M), and inferior (I) turbinates. In both drawings, the thick line along the surface of the nasal cavity is where olfactory epithelium is found. In the rabbit the olfactory epithelium is much more extensive. (Farbman, 1992 p. 17)
Figure 2. The basic diagram of the primary olfactory pathway is the bipolar sensory cell, with a cell body and dendrite in the periphery. The dendritic terminal contains fluid- or mucus-bathed tiny appendages that have access to odorants from the outside world. The axon enters the central nervous system to terminate on a synapse with a secondary neuron. The secondary neuron, in turn, projects its axon to other regions of the central nervous system. (Farbman, 1992, p. 4)
Figure 3. Diagrammatic illustration of the olfactory mucous membrane. The epithelium and lamina propria are shown. The long cilia on the surface are matted in a layer of mucus on the epithelium surface and lie parallel to the surface. Within the lamina propria are Bowman’s glands (BG), bundles of olfactory nerve processes (N), and blood vessels, both small arteries (A) and veins (V). Ducts from the BG open onto the surface. For clarity, the numbers of olfactory nerve bundles and cell bodies from which they originate are understated in this diagram. (Farbman, 1992, p25).
Figure 4. Known and proposed pathways of olfactory transduction. a) Generalized diagram of a primary olfactory receptor neuron. Olfactory transduction in vertebrates occurs in the olfactory cilia, which extends from the olfactory epithelium into a fluid layer that is exposed to the odour environment. b) Composite schematic diagram summarizing the two major second-messenger pathways implicated in olfactory transduction. One pathway, which is more completely understood, involves a receptor protein (R₁), a GTP-binding protein (G₁), and adenylate cyclase (AC) that produces cAMP (in bold), and a cation channel that is gated directly by cAMP (CNG; not labeled on figure). The other pathway involves a different receptor protein (R₂), a different GTP-binding protein (G₂), a phospholipase C (PLC) that produces InsP₃ (in bold) and diacylglycerol (DAG), and a cation channel that is directly gated by InsP₃. Each pathway can target more than one ion channel. Other ion channels implicated in the olfactory transduction include a Cl⁻ selective channel, a K⁺ selective channel, and a channel that is gated directly by InsP₄, which is produced most directly by the action of a protein 3-kinase (PK₃) on InsP₃. Each pathway also can be modulated by a number of regulatory elements. Regulatory elements implicated in olfactory transduction include a phosphodiesterase (PDE), a G-protein-coupled receptor kinase (GRK), protein kinase A (PKA), and protein kinase C (PKC), and Ca²⁺/calmodulin (CAM). This diagram shows all known and proposed signaling pathways; all pathways do not necessarily occur in each species. Solid, shaded arrows represent established pathways. Dashed arrows represent proposed pathways. (Figure and caption from Ache & Zhainazarov, 1995)
Figure 5. Diagram illustrating how odors are introduced to the nasal cavity and the steps the stimuli signals take to reach the brain. (Pszczola, 2004)
Figure 6. Transformation of odorant receptor inputs in the nervous system. The odor stimulates a neuron that passes information to glomeruli in the olfactory bulb. This information is filtered and sent to the appropriate location in the olfactory cortex so that information can be relayed as to what or how intense the aroma is. (Figure and modified caption from Zou et al., 2001)
Figure 7. Paramedian section of the human head showing the ortho- and retronasal routes of aroma perception.
Figure 8. Difference in sniffing and breathing in.
http://www.macalester.edu/psychology/whathap/UBNRPSmell/nasal.html
Figure 9. The taste bud (Hoon et al., 1999)
Figure 10. Functional anatomy of the tongue. Diagram of the human tongue, highlighting the regional preferences to sweet, sour, bitter, and salty stimuli. Note that while different areas of the tongue display strong preference to certain taste modalities, there is significant overlap between the various regions. Also shown, in expanded scale, are the three different types of taste papillae and their corresponding topographic distribution (for simplicity, taste buds were only drawn in one side of the papillae folds). (Hoon et al., 1999)
Figure 11. The velum (soft palate) and tongue
Figure 12. Summary of perceptual interactions evoked during ingestion. Arrowhead indicates a modality that has been demonstrated to interact with another modality. (figure and caption Delwiche, 2004)
<table>
<thead>
<tr>
<th>Factor</th>
<th>Cause</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Mastication</td>
<td>Increased surface area and mouth movements</td>
<td>-Increase release of aroma compounds</td>
</tr>
<tr>
<td></td>
<td>Possible in-mouth generation of volatiles (enzymes)</td>
<td>-Affects mouthfeel</td>
</tr>
<tr>
<td>Saliva</td>
<td>Interaction with saliva components (salts, enzymes)</td>
<td>-Affects release of aroma compounds</td>
</tr>
<tr>
<td></td>
<td>Dilution</td>
<td>-Possible phase inversion (saliva + temperature + shear) affects the release of aroma compounds and texture</td>
</tr>
<tr>
<td></td>
<td>Hydration</td>
<td>- Affects the release of aroma compounds and texture</td>
</tr>
<tr>
<td>Diffusion</td>
<td>Odorant and tastant release to saliva and air phase</td>
<td>-Affects the release of aroma compounds</td>
</tr>
<tr>
<td>Binding</td>
<td>Odorant and tastant to the mucosa</td>
<td>-Affects the release of aroma compounds</td>
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<tr>
<td></td>
<td></td>
<td>(especially hydrophilic compounds)</td>
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<tr>
<td>Temperature</td>
<td>Changes volatility of odorants</td>
<td>-Affects the release of aroma compounds and texture</td>
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<td></td>
<td>Melting</td>
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(Taken from Miettinen, 2004)
Review of Literature

Part II. Beef Flavor

Introduction

Sixty-seven percent of the variation in overall beef palatability from consumer in-home studies can be attributed to flavor (Huffman, Miller, Hoover, Wu, Brittin, & Ramsey, 1996) with 39-40% of consumers rating flavor as the most important attribute for beef palatability (Huffman et al., 1996; Miller, Huffman, Gilbert, Hamman, & Ramsey, 1995). While arguments may arise on which attribute (flavor or tenderness) is the most important to overall beef palatability, flavor is vital in ensuring a desirable eating experience.

Hornstein, Crowe, & Sulzbacher (1960) started investigating naturally occurring substances that give beef its flavor. They found the main flavor precursors were water-soluble and when the water soluble portion was concentrated and heated, the powder developed a flavor similar to cooked beef. When water-extracted ground beef was cooked, it was tasteless and odorless. In addition to the water-soluble portion, an oily, viscous, liquid solution with a low vapor pressure also possessed a strong aroma. Several decades have passed, and the question of the compounds contributing to beef flavor still exists although progress is being made with the advancement of technology, especially with gas chromatography (GC) and mass spectrometry (MS).

Nearly one thousand compounds have been found in the volatile portion of meat, but determining which compounds contribute and/or interact with other compounds to create desirable or undesirable flavor is still relatively unknown. Hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, ethers, furans, pyridines, pyrazines,
pyrroles, oxazoles, oxazolines, thiazoles, thiazolines, thiophenes, other sulfur compounds, and halogen containing compounds make up the volatile portion of cooked beef (Shahidi, 1989) with mercaptothiophenes and mercatofurans contributing significantly to beef aroma (MacLeod, 1986). By 1998, twenty-five compounds had been identified as possessing ‘meaty’ aromas (Figure 1). Most of the volatiles contributing to normal beefy flavor are sulfur-containing compounds.

Raw meat has little aroma and a blood-like taste (Crocker 1948; Bender & Ballance, 1961). Interactions between volatile compounds, nonvolatile compounds (free amino acids, peptides, reducing sugars, vitamins, and nucleotides), and lipids via Strecker degradation, Maillard reactions, thermal processing and/or oxidation develop the overall flavor of beef. Diets (direct transfer from feeds to tissue), metabolic pathways, enzymatic reactions, and species also play a role in the perceived flavor and volatiles of meat by the consumer (Vasta, & Priolo, 2006). Mottram (1998) divided flavor precursors into two major categories: water soluble components and lipids. Shahidi (1998) also broke down the nonvolatile aroma components into three parts: lipid oxidation, thermal degradation and the resulting interactions, and thermal degradation of thiamin.

**Beef Species Flavor**

When studying pork and beef flavor precursors, Hornstein & Crowe (1960), determined pork and beef have similar, basic, meaty flavors in the lean tissue that appeared to be low molecular weight, cold water-soluble compounds. They hypothesized the compounds interacted with amino acids, carbohydrates, and polypeptides to produce the flavor of lean meat. During the same time period, Batzer, Santoro, Tan, Landmann, & Schweigert (1960) used column chromatography and gel filtration to also conclude
unknown, low molecular weight, water-soluble compounds, basic amino acids, carbohydrates, peptides, and phosphates were precursors to beef odor. This established cooked meat flavor was not a single compound or a class of compounds. Hornstein & Crowe (1960) also found pork and beef had different free fatty acids and carbonyls and different concentrations of fatty acids and carbonyls that produced different volatiles when heated which suggested that flavor differences in species was due to the fat portion.

**Live Animal Factors**

*Heritability*

Splan, Cundiff, & Van Vleck (1998) looked at 2,386 animals with 577 sires and found that taste panel flavor ratings had a 0.04 estimate of heritability which was not significantly different from zero. Other studies support these data that taste panel flavor scores of beef steaks are not inherited from sires or dams (Wilson, McCurley, Ziegler, & Watson, 1976; Van Vleck, Hakim, Cundiff, Koch, Crouse, & Boldman, 1992). Further modeling with 2,360 records determined the variance (0.93±0.06) of beef’s taste panel flavor was mainly due to environmental effects (Nephawe, Cundiff, Dikeman, Crouse, & Van Vleck, 2004). This is in agreement with Shahidi & Rubin (1986) that feed source is the most important environmental factor affecting meat flavor.

*Animal Gender*

The effect of testosterone on beef flavor has conflicting results in different studies (Paterson, Jones, Gee, Costello, & Romans, 1987; Hawrysh, Price, & Berg, 1979; Forrest, 1975; Field, 1971; Reagan, Carpenter, Smith, & King, 1971; Field, Helms, & Schoonover, 1966), but the conflicting results seem to mainly be due to the age of the animal when slaughtered. Several hypotheses have been given to explain the possible
impact on flavor based on sex of the animal. Testosterone increases muscle growth and decreases lipid deposition so meat-like flavor increases and fat-associated flavor decreases (Miller, 2001). Intact males also are more likely to have higher myoglobin content and dark, firm, and dry characteristic meat which has a higher pH. Reagan et al. (1971) determined steaks from bulls acquire undesirable flavor between the ages of 385 d and 484 d while Field et al. (1966) showed heifer and steer meat were similar to bull meat until animals reached over 600 d of age. With pH higher than 5.6-5.9, meat is described as musty/moldy, more intense beef flavor, cowy/grainy, and/or serumy/bloody. Higher myoglobin levels in bull meat have been suggested to lead to greater sensations of metallic, liver, serumy/bloody, and bitter flavors (Miller, 2001). Beef from bulls was found to have higher livery odor and flavor and bloody flavor than heifers which were found to be related to higher 2-propanone levels using multiple regression and discriminant analysis (Gorraiz, Beriain, & Insausti, 2002). Reagan et al. (1971) found steaks from steers 385 d old were approximately 86% likely to be desirable to in-house panelists in flavor compared to approximately 32% for steaks from bulls at the same age. Once steers reached 484 d of age, the flavor of steaks were only 36% desirable compared to 27% from bulls.

When sensory traits from fed and non-fed beef and dairy cow muscles were compared to A-maturity USDA Select steer muscles, no differences were seen between the cow groups (Stelzleni, 2006). However, the Select muscles from steers were found to have lower beef flavor intensity than the cow muscles (5.5 versus 5.7, respectively, out of an 8-point scale).
When meat from bulls and steers were used in restructured products, there were no differences in any palatability traits evaluated by trained panelists (Paterson et al., 1987). Because the bull meat was leaner, the restructured steaks were less prone to oxidative rancidity than restructured steaks from finished steers (Paterson et al., 1987). When slaughter age (16-17 mo), breed, background diet, and finishing diet were held constant, steers, intact bulls, and short scrotum bulls demonstrated no difference in flavor scores (Albaugh, Carroll, Ellis, & Albaugh, 1976).

In 344 steers and 302 heifers, no differences (6.1 ± 0.7 and 6.2 ± 0.07, respectively) were observed in taste panel flavor scores (Wilson et al., 1976). In contrast, Hood and Allen (1971) found aroma differences between cooked beef from 14-mo, half sibling heifers and bulls which they attributed to fatty acid compositional differences and/or to the different free fatty acids in the intramuscular lipid.

**Animal Age**

Numerous beef studies have indicated the decrease in desirability of palatability traits, especially tenderness, as carcass maturity increases (Miller, 2001; Boleman, Miller, Buyck, Cross, & Savell, 1996; Miller, Tatum, Cross, Bowling, & Clayton, 1983; Berry, Smith, & Carpenter, 1974). In terms of the impact of animal age on flavor, Field et al. (1966) found that animal age was positively correlated (0.36) to flavor in steers and heifers which means that older steers and heifers (study compared 300-700 d animals) were more palatable than younger steers and heifers. Bull meat flavor was not correlated to the ages tested in that study, but after 600 d of age, meat from bulls was significantly different in flavor from steer and heifer meat. Smith, Savell, Cross, & Carpenter (1983) found a significant decreasing linear trend with increasing carcass maturity (A-E) to
flavor desirability. Jacobson and Fenton (1956) found a decrease in flavor acceptability of meat from heifers older than 336 d. Increasing the age of bulls and steers by 100 d decreased the percentage of steaks rated as desirable (31.8% to 26.1% and 85.7% to 36.4%, respectively) while undesirable flavor scores raised from 4.7% to 27.2% in steers and 22.7% to 52.2% in bulls (Reagan et al., 1971).

In order to improve the palatability of meat from older animals, supplemental feeding has been investigated since most mature beef animals are sold after coming off pasture. Miller et al. (1983) found no difference in beef flavor desirability between A/B maturity and C/D maturity carcasses after the animals in the study were finished as a group on a high-energy grain diet. When mature cows were feed a high energy diet longer than 28 d, the flavor intensity of the meat was greater, while off-flavor scores slightly decreased (Boleman et al., 1996).

In the fall of 2003, the University of Florida and University of Nebraska began a benchmarking study to investigate the differences between fed and non-fed beef and dairy cows with A-maturity, USDA Select steers (Stelzleni, 2006). No difference was found for flavor intensity of the cow groups, but the USDA Select carcasses had meat with slightly lower flavor intensity than the cow populations. The muscles from the beef non-fed cow group had the most off-flavors while the A-maturity, Select muscles had the least. The beef non-fed population was the oldest maturity group and most likely had come to the slaughter plant without supplemental high-energy feed which would explain why they had the highest off-flavor scores.
**Diet**

The primary focus on the effect of diet and flavor acceptability has been comparing pasture-fed animals to grain-fed animals. A wide range of results have been reported; some papers suggesting there is no difference in forage-fed animals and others stating there are large differences. Most of the differences can probably be explained by the different production systems which affect the level of energy intake, days on feed, growth rate, age of the animal, fat deposition, fat composition, and carcass weight. Additionally, Brown, Melton, Riemann, & Backus (1979) stated sensory panels do not find a lack of flavor in grass-fed beef, ‘but rather the presence of an off-flavor.’

Compared to same age steers fed corn silage-, pasture-, and Bermuda pellets, steers finished 90 d on high energy corn based diets had more desirable or intense beef flavor (Melton, 1983). When feeding to a constant fat thickness in different production management systems, flavor differences existed (Bowling, Riggs, Smith, Carpenter, Reddish, & Butler, 1978). Even when comparing corn diets to corn silage diets, significant differences were seen in flavor profiles of beef, although not to the extent of grass or alfalfa finished steers (Berry, Leddy, Bond, Rumsey, & Hammond, 1988). In contrast, when animals were blocked by growth rate, no difference in flavor was seen between the grass- and grain-fed animals (French et al., 2001). The high growth rate animals fed on grass had little difference in meat quality to concentrate fed cattle which the authors attributed to high protein turnover (French, et al., 2001).

Several grasses in ruminant diets have been demonstrated to cause less desirable meat flavor including, Flint hills pasture in Kansas, orchard grass-clover, rye-oats-ryegrass, forage sorghum, bluegrass-clover, fescue, fescue-orchard grass-clover, rye-
ryegrass-clover, arrow leaf clover, bermuda-clover-sudan, millet, and coastal Bermuda grass (Melton, 1990). In contrast, Bidner, Montgomery, Babley, & McMillin (1985) found no difference in flavor intensity in meat from animals fed high quality bermudagrass pasture compared to corn-based diets although electrical stimulation, blade tenderization, and aging were also variables in this study and might have confounded the results. French et al. (2000a) found similar results after aging the meat 2 d when steers were finished on autumn grass, grass silage, or concentrate diets with low levels of supplements to maintain constant carcass growth rate between treatments. Melton (1983) suggested differences in results could be due to differences in sensory panels or quality of the grasses.

Hay diets were also found to produce meat less desirable in flavor than corn silage diets with no direct link to intramuscular fat (Dube et al., 1971), while another study showed the opposite effect (meat from animals on a 91% corn diet were less desirable in flavor than meat from animals fed alfalfa or timothy hay) when using hay as the energy source (Oltjen, Rumsey, & Putnam, 1971). Furthermore, hay versus grass silage diets fed at the same net energy do not affect flavor (Listrat, Rakadjiyski, Jurie, Picard, Touraille, & Geay, 1999). Melton (1983) concluded corn could be replaced partially or totally with high quality alfalfa or in combination with timothy hay and not see a significant change in flavor.

Corn is the staple grain used in grain-fed cattle in the USA while Canada and Japan use barley. No differences in flavor intensity as determined by a trained flavor and descriptive panel were found when comparing corn, barley, and 50-50 corn/barley diets in the meat from young animals (Miller, Rockwell, Lunt, & Carstens, 1996).
Additionally, 12 aromatics, two mouthfeels (astringent and metallic), and three tastes were not found to be different in muscle samples from the three diets. In contrast with a consumer panel, Sitz, Calkins, Feuz, Umberger, & Eskridge (2005) and Jeremiah, Beauchemin, Jones, Gibson, & Rode (1998) found USA consumers preferred the flavor of domestic beef over Canadian barley fed beef.

The majority of the flavor effect due to feeding of forages is hypothesized to be due to changes in lipid deposition and fatty acid composition. Using sheep as a ruminant model, Lee, Winters, Scollan, Dewhurst, Theodorou, & Minchen (2004) hypothesized red clover fed to grass-finished steers would increase both \( n-6 \) and \( n-3 \) polyunsaturated fatty acids (PUFA) due to reductions in ruminal biohydrogenation of PUFA caused by polyphenol oxidase’s protective attributes as it did in ovine study. French et al. (2000b) found meat from cattle that were grass supplemented to maintain constant growth rate with concentrate-fed animals had a linear decrease in saturated fats and \( n-6:n-3 \) PUFA ratio and increase in unsaturated fats and conjugated linoleic acid (CLA) when concentrate percentage went down, without affecting flavor scores (French et al., 2000a). Fishy, bloody, and overall flavor liking scores were significantly different in meat from grass-finished animals with increased \( 18:1trans \) isomers and, notably, \( \text{CLA}_{cis-9, trans-11} \) (Nuernberg et al., 2005). Animals backgrounded on grass and then finished approximately 190 d on a high energy diet of silage, hay, and barley had meat with higher levels of \( n-3 \) fatty acids than animals fed concentrate after weaning, but no difference in \( \text{CLA}_{cis-9, trans-11} \) (Dannenberger et al., 2004) were found in the lipids of the longissmus muscle and subcutaneous fat (Dannenberger, Nuernberg, Nuernberg, Scollan, Steinhart, & Ender, 2005). However, \( \text{CLA}_{trans-7, cis-9} \) was the second most abundant
CLA isomer in meat from concentrate-fed animals whereas CLA\textit{trans}-11, \textit{cis}-13 was the second most abundant in grass-fed. Total CLA isomers were increased in the longissus, subcutaneous fat, heart, and liver, but not in the semitendinosus (Dannenberger et al., 2005) in grass-fed animals. Most importantly, this study showed \textit{\Delta}^9\textit{-desaturase} activity was decreased due to pasture feeding. This elongase, in conjunction with \textit{trans} vaccenic acid, is responsible for the synthesis of CLA\textit{cis}-9, \textit{trans}-11. By disrupting the elongase activity flavor changes might occur because of the unused \textit{trans} vaccenic acid, a fatty acid implicated in off-flavors (Camfield, Brown, Lewis, Rakes, & Johnson, 1997) as well as less CLA\textit{cis}-9, \textit{trans}-11 (Dannenberger et al., 2005). French et al. (2000b) also hypothesized the increase in CLA\textit{cis}-9, \textit{trans}-11 in animals on higher grass-based diets, when ingestable 18:2 was held constant for all treatments, was also due to a change in biohydrogenation. However, they concluded grass diets favored the growth of \textit{Butyrivibrio fibrisolvens}, the ruminal bacterium responsible for producing the linoleic acid isomerase.

With increased interest to increase the PUFA in beef, trials with supplements high in certain fatty acids have been conducted. Most attempts have been made using linseed, linseed oil (slight increase in 18:3\textit{n}-3; decrease in \textit{n}-6:\textit{n}-3 PUFA ratio), sunflower, sunflower oil (increase 18:2\textit{n}-6; increase \textit{n}-6:\textit{n}-3 PUFA ratio), and fish oil (increase 20:5\textit{n}-3 and 22:6\textit{n}-3) (Scollan, Hocquette, Nuernberg, Dannenberger, Richardson, & Moloney, 2006; Mandell, Buchanan-Smith, Holub, & Campbell, 1997). Most studies have not reached high enough levels of PUFA to claim health benefits, but some negative flavors due to oxidation and shorter shelf-life have been reported (Miller, 2001). The long chain fatty acids in fish oil (Richardson, et al., 2004) and several long chain fatty
acids from plant oils can bypass rumen biohydrogenation with minimal change (Scollan et al, 2004). This increase in unsaturation can lead to negative flavor perception.

After 80-90 d on a corn finishing diet, no further significant beef flavor changes occur (Melton, Black, Davis, & Backus, 1982). Liver flavor intensity increased up to 86 d of corn based diets, and sour flavor intensity decreased to a minimum at 122 d on corn, while metallic and off-flavor intensity were unaffected by time-on-feed. Fishy and milky-oily linearly decreased with time-on-feed. Melton et al. (1982) hypothesized increased beef fat and liver flavor with decreased milky-oily, sour and fishy flavor gave a more desirable beef flavor in corn-fed beef. Mandell, Buchanan-Smith, & Campbell (1998) disagreed with this hypothesis as they found liver flavor was positively correlated to metallic and grassy aroma, sour flavor, and metallic and grassy aftertaste and negatively correlated to beef flavor. They also found sour was not affected by production type, but metallic aroma was affected due to the differences in 18:1 and 18:3 in the meat from the different feed sources.

The biggest difference in the flavor of meat from grass- and grain- fed beef animals probably is due to fatty acid concentration and type as fatty acids are the primary source of carbonyl compounds (Melton, 1983). Oleic and linoleic acid are found in higher concentrations in grain-fed diets than in grass-fed diets (Vasta et al., 2006; Enser, Hallett, Hewitt, Fursey, Wood, & Harrington, 1998) while α-linolenic is higher in grass-based diets (Enser et al., 1998). Therefore, compounds which are derived from linolenic acid, 4-heptenal, 2,4-heptadienal, and 2,6-nonadienal, are usually in higher concentration in meat from grass-fed animals while hexanal, 2-heptenal, and 2,4-decadienal (products of linoleic acid) are typically found in higher concentrations in meat from grain-fed
animals (Larick et al., 1987). Furthermore, beef from corn-based diets have higher levels of glucose (Melton, Black, Davis, & Buckus, 1982; Brown et al., 1979), γ and α-tocopherol (Yang, Lanari, Brewster, & Tume, 2002), and carotenoids (Melton, 1990; Yang et al., 2002).

Larick et al. (1987) investigated differences in volatile compounds in forage systems and grain fed animals. Fat from animals grass-finished on tall fescue, brome grass-red clover and orchard grass-red clover pastures were not different in volatile compounds, but 31 volatiles were in different concentration in the meat from grain-fed animals. These volatiles that were higher in the meat fat from grass-fed animals include pentanoic, heptanoic, octanoic, nonanoic, decanoic, and dodecanoic acids; heptanal, 2,3-octanedione, 3-hydroxy-octan-2-one, 2-decenal, 2-tridecanone, hexadecane, heptadecane, octadecane, δ-dodecalactone, phyt-1-ene, neophytadiene, phyt-2-ene, an isomer of neophytadiene, 2-heptadecanone, dihydrophytol, and phytol with the terpenoids in much higher concentration due to rumen fermented chlorophyll (Suzuki & Bailey, 1985). The fat from the grain-produced animals was higher in δ-tetradecalactone and δ-hexadecalactone (Larick et al., 1987). These lactones are derived in the rumen by the oxidation of linoleic and oleic acids (Vasta et al., 2006). In the study, Larick et al. (1987) found phyt-2-ene to be highly correlated to beef flavor intensity while δ-tetradecalactone and δ-hexadecalactone were negatively correlated to grassy flavor. Pentanal, toluene, 1-ethyl-2-methylbenzene, and an unknown compound explained 51% of the variation of beef fat flavor intensity between grass and grain finished (Melton, 1990). As days on feed increased, pentanal, hexanal, 4-methyl-3-penten-3-one, nonane, acetone, nononal, and two unknown compounds increased while trans-3-octene, cis-2-octene, toluene, 3-
penten-2-one, 3-hydroxy-2-butanone, and five unknown compounds decreased (Melton, 1990).

Several classes of compounds are affected by the animal’s diet. Descalzo et al. (2005) found more aldehydes in meat from animals eating concentrate diets rather than grass. Many typical beef flavor compounds are aldehydes so one would expect to see an increase in aldehydes to produce the recognizable flavor of cooked beef. Phenolic compounds are secondary metabolites of plants so they are typically found in higher concentration in meat of forage-finished animals compared with grain-finished with the exception of 4-ethylphenol and cresols (Vasta et al., 2006). Diets play a large role on indoles and their derivatives with grass-fed animals having much higher levels, especially of skatole. Production of these indoles from ruminal microorganisms can be reduced by feeding feedstuffs with higher levels of tannins for a few d (Vasta et al., 2006). The volatile 2,3-octanedione has been suggested as an indicator of grass-fed animals since the compound is produced by a lipoxygenase on leafy plants (not seeds) (Young, Berdague, Viallon, Rousset-Akrim, & Theriez, 1997) and soybeans (Elmore, et al., 2004). This compound can also be derived from heating and breaking down linoleic acid (Elmore, Campo, Enser, & Mottram, 2002) so care is needed if using the compound as an indicator of grass-fed animals. Terpenoids are directly transferred from grass to animal tissue so these compounds are also considered a green forage indicator except for β-gurjunene and limonene, which are higher in concentrate-fed animals (Vasta et al., 2006). Cornu, Kondjoyan, Fencia, & Berdague (2001) discovered several terpenoids, including β-pinene, in beef could be used to determine the region that an animal came from based on volatile compounds from the forages in the geographic area that were ingested by the
animal. Because of sulfur’s low threshold, the small amount of these volatile compounds in meat plays a significant role in meat flavor (Drumm, & Spanier, 1991) with aldehydes from PUFA playing a role in the synthesis of these heterocyclic compounds (Vasta et al., 2006). Typically, sulfur compounds are in higher concentration in the grass-fed animals because of the sensitivity of the fatty acids to convert to aldehydes during thermal processing (Elmore, Mottram, Enser, & Wood, 1999). There has also been some thought the higher ultimate pH in meat from grass-fed animals might favor the formation of thiazoles and thiophenones because of the availability of amino acid degradation products while decreasing other sulfur volatiles that favor lower pH (furanthiols, mercaptokin, aliphatic sulfides, and thiopenes: breakdown products of cysteine).

The source of feed can play a role in the oxidative stability of beef. When cattle were finished on a mixed diet of silage, hay, and concentrate (corn, beet pulp, and linseed cattle-cake), thiobarbituric acid reactive substances (TBARS) were always significantly higher than grass fed animals regardless of age of animal or storage condition (Gatellier, Mercier, Juin, & Renerre, 2005). Brown et al. (1979) also found ground beef from steers on low energy diets had more free fatty acids and lower TBARS values than meat from animals that consumed a high energy diet. This was attributed to the increased levels of vitamin E in biological membranes and fat of grass-fed animals although it was noted the grain diet also contained antioxidants of proanthocyanidins and phytic acid (Gatellier et al., 2005). In the same study, a higher heme iron content (considered to be a prooxidant) was found in the heifer and cow meat on the mixed diets compared to the grass diets and the steers, which they concluded also affected the increased oxidation. Interestingly,
when grain-fed animals are supplemented with vitamin E, the same level of tocopherol is achieved in the lean tissue, and the meat is more stable following 47 d vacuum packaged storage than grass-fed beef with or without supplementation. Therefore, 4-6 μg/g of α-tocopherol in the meat of supplemented grain-fed animals is adequate to minimize lipid oxidation, but not in grass-fed beef (Yang et al., 2002).

It is important to note most of these findings on flavor were studied in the United States. An individual usually comes to prefer the foods he/she grew up eating. Sitz et al. (2005) and Killinger, Calkins, Umberger, Feuz, & Eskridge (2004) found the greatest sensory difference between Australian or Argentine (respectively) grass-fed and USA grain-fed beef to be flavor when Warner-Bratzler Shear force values were kept constant. Canadian, barley-finished cattle were also rated less desirable for flavor than domestic beef (Sitz et al., 2005). However, 19% of the consumers in the study preferred the Australian meat when compared to domestic beef while 29.3% preferred the Canadian-fed beef when compared to domestic beef. Consumers in both studies were willing to pay a premium for their preference, which was heavily influenced by flavor.

**Aging**

Post-mortem aging has been widely studied to determine the how tenderness is affected by storage time after slaughter. Aging has also been found to have a profound effect on flavor. Spanier, Flores, McMillin, & Bidner (1997) discovered desirable flavors of beefy, brothy, browned-caramel, and sweet start to gradually decline after 4 d post-mortem while bitter, sour, painty, and cardboardy increase in intensity at a moderate rate. In fact, top round meat was found to have ‘optimum flavor’ at 4 d post-mortem in vacuum packaging; the authors speculated the decline with additional aging was due to
peptide production caused by calpain proteases (Koohmaraie, Babiker, Merkel, & Dutson, 1998) and/or cathepsins (Spanier, McMillin, & Miller 1990). Monson, Sanudo, & Sierra (2005) found there was no breed, aging, or breed by aging interaction for beef flavor intensity, liver flavor intensity, or liver odor intensity. Several studies have found aging affects most flavor attributes including overall odor intensity, liver intensity, overall flavor intensity, acid flavor intensity (sourness), and liver flavor intensity (Smith, Culp, & Carpenter, 1978; Miller, Kerth, Wise, Lansdell, Stowell, & Ramsey, 1997; Campo, Sanudo, Panea, Alberti, & Santolaria, 1999). However, Monson et al. (2005) did see a significant effect due to aging on beef odor intensity (peaked at d 21) and bitter flavor intensity (linear increase). As was seen in Campo et al. (1999) and Monson et al. (2005), the highest odor intensity was approximately 21 d age. Additionally, after 10 d postmortem, there was a gradual decline in beef flavor (not significant), but a significant increase in undesirable, bitter, aromatic flavors (Monson et al., 2005; Spanier, et al., 1997). Miller et al. (1997) found there was actually an increase in flavor intensity between 7 and 14 d aging with no quality grade by aging interaction for flavor intensity; there was a main effect of quality grade for flavor intensity, with USDA Choice having higher flavor intensity than USDA Select. Meat from the beef breeds required less aging time to reach optimum flavor and palatability scores than dairy or dual purpose breeds (Monson et al., 2005). Enhancement has also been shown to reduce the aging time necessary to increase tenderness and juiciness while inhibiting the development of metallic flavors (Wicklund, McKeith, & Brewer, 2003). Monson et al. (2005) concluded flavor is improved during aging, but reaches an optimum level before off-flavors begin to develop such as rancid and fatty (Gorraiz et al., 1991).
Aging can be done by dry aging (meat is left in a cooler with controlled humidity) or by wet aging (meat is sealed in a vacuum bag and held slightly above freezing temperatures). Conflicting results are reported for the effect of aging on flavor development (Campbell, Hunt, Levis, & Chambers IV, 2001; Parrish, Boles, Rust, & Olsen, 1991; Bischoff, 1984). Sitz, Calkins, Feuz, Umberger, & Eskridge (2006) found consumers did not find differences in USDA Choice wet- or dry-aged steaks, but did find flavor and other sensory differences in USDA Prime wet- or dry-aged steaks. Consumers preferred the wet-aged USDA Prime steaks. Campbell et al. (2001) found higher beef flavor intensity, dry-aged flavor, and brown roasted aromas in the 14 and 21 d dry aged steaks compared to the 14 and 21 d wet-aged steaks. There is also a possibility the dry-aged steaks developed more off-flavors during the aging period than the wet-aged due to contact with air (oxygen).

Flavor compounds and flavor intermediates are developed during aging that can react to form other flavors during cooking (Maillard reaction). Aging affects sugars, organic acids, peptides, free amino acids, metabolites (ATP), enzyme location in intracellular compartments, and enzyme activity - all of which play a role in flavor development (Gunther, & Schweiger, 1966; Dannert, & Pearson, 1967; Parrish, Goll, Newcomb, deLumen, Chaudhry, & Kline, 1969; Hood et al., 1971; Spanier et al. 1990). Aliphatic hydrocarbons, mainly branched alkanes produced by oxidation, develop during aging from 2 to 7 d (Gorraiz et al., 2002). However, Hood et al. (1971) suggested aging does not cause autoxidation since no effect was seen on the intramuscular phospholipid fraction of meat.
Muscles

Most of the research comparing muscles has dealt with tenderness because there is approximately 3-4 times the variation in tenderness compared to flavor (Shackelford, Wheeler, & Koohmaraie, 1995; Wulf, & Page, 2000) especially in the M. longissimus dorsi. Table 1 and 2 list the rankings of muscles for flavor intensity and off-flavor intensity from various studies. The muscle that is ranked first had the highest flavor intensity or lowest off-flavor score. In most of the studies the difference in beef flavor intensity between muscles was less than 1.5 units although it varied due to scales used. Because of the wide range of muscles tested it is difficult to draw many conclusions on which muscle has the highest beef flavor intensity.

Beef flavor intensity was correlated to off-flavor intensity (r=0.71) and weakly correlated to all other traits (tenderness, r=-0.14; amount of connective tissue, r=-0.11; juiciness, r=0.13; sarcomere length, r=-0.31; percentage of desmin degraded, r=0.34; cooking loss, r=-0.20) except collagen concentration and shear force (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004). When simple correlations were run for each individual muscle, all the muscles in the study only had significant correlations with off-flavor intensity, except the infraspinatus’s correlation to collagen concentration (r=-0.38) and the longissimus correlation to juiciness (r=0.44). In contrast, Jeremiah, Dugan, Aalhus, & Gibson (2003) found no correlations for flavor intensity.

Meisinger, James, & Calkins (2006) found the M. infraspinatus had the least off-flavors and the lowest frequency of sour notes of the six chuck and round muscles tested. The M. vastus medialis had the most intense off-flavor ratings with a high frequency of sour, charred, and oxidized flavor notes. Liver-like, bloody, rancid, and heme iron were
not affected by muscle. When samples were divided into two groups based on sensory evaluations for liver-like flavor notes, there were no differences for sour, metallic, bloody oxidized or fatty off-flavors between the groups. The *M. rectus femoris*, *M. teres major*, *M. vastus lateralis*, and *M. vastus medialis* demonstrated a relationship with pH, heme iron and off-flavor intensity, although pH and heme were not related to specific off-flavor notes.

Flavor desirability has been used by some researchers in addition to or in lieu of flavor intensity. The Canadian Cattlemen’s Association established a goal of 95% consumer acceptance of beef, and seven muscles or muscle groups—*M. teres major*, *M. psoas major*, *M. longissimus thoracis*, *M. longissimus lumborum*, *M. ilio psoas*, *M. spinalis dorsi*, and *M. subscapularis*—fell into that category for flavor desirability (Jeremiah, Gibson, Aalhus, & Dugan, 2003). However, the two longissimus muscles were rated as the second and third lowest for beef flavor intensity although the range for flavor intensity was 5.00 to 6.07 on a 9-point scale in that study. Five other muscles or muscle groups were approaching 95% desirability for flavor as well. McKeith, De Vol, Miles, Bechtel, & Carr (1985) found the *M. psoas major*, *M. infraspinatus*, *M. longissimus thoracis*, *M. longissimus lumborum*, and the *M. rectus femoris* to be rated significantly higher than *M. supraspinatus*, *M. semimembranosus*, *M. semitendinosus*, *M. adductor*, and *M. pectoralis profundus* for flavor desirability with the *M. biceps femoris*, *M. gluteus medius*, and *M. triceps brachii* being similar to the two groups. Similar findings from Wulf et al. (2000) revealed the *M. longissimus dorsi* and *M. gluteus medius* had the same mean for flavor desirability (5.73 with 8 = intense) while the *M. semimembranosus* was less desirable. Flavor desirability was highly, negatively
correlated ($P < 0.001$) to insoluble collagen in a study that analyzed 33 muscles or muscle groups from 25 Canada AA steer carcasses (Jeremiah et al., 2003). Wulf et al. (2000) revealed flavor desirability was related ($r=0.61, 0.62, \text{ and } 0.26$ for $M. \text{ longissimus dorsi}$, $M. \text{ gluteus medius}$, and $M. \text{ semimembranosus}$, respectively) to an overall palatability index. This study also showed a quadratic relationship with increasing $L^*$ color values for the $M. \text{ longissimus dorsi}$ and gluteus medius, a linear relationship with increasing $b^*$ color values and pH for two muscles. Additionally, seven, six, and one carcass traits were correlated to beef flavor desirability including $L^*$, $b^*$, and pH for the $M. \text{ longissimus dorsi}$, $M. \text{ gluteus medius}$, and $M. \text{ semimembranosus}$, respectively. Beef flavor intensity was not correlated to as many carcass traits (three, two, and one) in the three muscles.

No muscle effect for flavor intensity was seen due to glycolytic potential, but less than 80 $\mu$mol/g affected the $M. \text{ longissimus dorsi}$ for flavor desirability while the $M. \text{ gluteus medius}$ saw a linear increase in flavor desirability with an increase in glycolytic potential (Wulf, Emnett, Leheska, & Moeller, 2002).

Cow muscles have very different flavor characteristics compared to meat from A-maturity steer carcasses. The benchmarking study compared sensory properties of muscles from Select, A-maturity carcasses to muscles from four cow populations (beef-fed, beef-nonfed, dairy-fed, dairy-nonfed) (Stezleni, 2006). Eight of the nine muscles rated for off-flavor in the beef-nonfed group were different from the Select group while only three of the nine muscles were different in the beef-fed group. The dairy-nonfed $M. \text{ latissimus dorsi}$ and $M. \text{ tensor faciae latae}$ were different from the Select, and the dairy-fed had three out of the nine different from Select. The $M. \text{ infraspinatus}$ was rated the
same as the Select *M. infraspinatus* for every cow population, except for the dairy-fed. The *M. longissimus dorsi*, *M. triceps brachii*, *M. rectus femoris*, and *M. teres major* were similar to the Select group for all cow populations except beef-nonfed. This was explained as a diet effect since the dairy-nonfed group probably received supplements in the diet while the other two groups probably received concentrate fed prior to harvest. There was no interaction in the study for group by muscle for flavor intensity. The *M. infraspinatus* received lower beef flavor intensity ratings compared to the other eight muscles.

**Maillard Reaction**

The Maillard reaction, or nonenzymatic browning, was introduced by Louis Maillard in 1912 to help explain amine and carbonyl reactions. Figure 2 illustrates the general schematic of the Maillard reaction. In general, amino compounds condense with a carbonyl group of a reducing sugar. This produces glycosylamine which is rearranged and dehydrated to form furfural, furanone derivatives, hydroxyketones, and dicarbonyl compounds. As the reaction progresses, the intermediates can react with other amines, amino acids, aldehydes, hydrogen sulfide, and ammonia.

**Amadori Rearrangement**

Mario Amadori (1929) discovered a condensation reaction that lead to one isomer that was more unstable and susceptible to hydrolysis and decomposition in air than a second isomer that was produced. Kuhn & Weygand (1937) later called this “The Amadori Rearrangement” after determining the rearranged compound was 1-amino-1-deoxy-2-ketose. The glycosylamine is rearranged into 1-amino-1-deoxy-2-ketose which can form two isomers that can give way to the rest of the Maillard reaction (Figure 3).
**Strecker Degradation**

Strecker degradation is the breakdown of amino acids and dicarbonyl compounds (Shahidi, 1998). To become aldehydes, the amino acids are decarboxylated and deaminated while the dicarbonyls become α-aminoketones or aminoalcohols. These aldehydes are condensed to aldols that form furans, pyrazines, pyrroles, oxazoles, thiazoles, and other heterocyclic compounds that are odor molecules.

**Schiff Base**

The Schiff base reaction is also called the furfural path where there is a loss of three water molecules followed by a reaction with amino acids and water. This product undergoes aldol condensation to become true melanoids (Manley, & Choudhury, 1999).

**Melanoidins**

Once the reaction has progressed through the Schiff base, Strecker degradation, or other pathways, the reactions can lead to melanoidins (brown, high molecular weight polymers from the condensation of cyclic compounds) (Fay, & Brevard, 2005). These products can be pleasing or unacceptable flavors and aromas (Manley et al., 1999).

**Product Compounds**

The states of different sugars and amino groups can produce different end products. Cysteine and glucose produce mainly sulfur compounds whereas cysteine and glucose under oxidized conditions produce more pyrazines and furans (Tai, & Ho, 1997). Maillard volatile compounds from glutathione and glucose produce sulfur-containing compounds- thiophenes, thiazoles, and cyclic polysulfides- at pH 6 and 8, but furans are more favorable end products at more acidic pH. When glutathione is oxidized it becomes glutathionesulfonic acid, which produces furans, carbonyls, pyrroles, and pyrazines with
glucose. Sulfur-containing compounds are not formed when glutathione is oxidized (Tai, & Ho, 1998).

Cysteine and ribose through the Maillard reaction as well as thiamin have been shown to create compounds such as 2-methyl-3-furanthiol (Mottram, & Whitfield, 1994). From these compounds thiols and disulfides can originate (Figure 4).

An exhaustive review of the nine most common aromatic compound classes from precursors of the Maillard reaction can be found in Manley et al. (1999).

\textit{pH}

The pH of food plays a role in the development of flavors in the Maillard reaction. As pH increases, color and polymeric compounds increase and nitrogen-containing compounds like pyrazines are favored (Mottram et al., 1994). Since fresh meat only has a pH range of around 5.5-6.0 with a good buffering ability, little work has been done to investigate the effect of pH on Maillard products although meat above the normal pH range is perceived to have a decrease in meat flavor intensity.

\textit{Fat}

Lipids serve several roles in flavor development. Lipids in meat act as a solvent for the volatile compounds that develop during production, handling, and thermal processing (Moody, 1983). They undergo thermal oxidative change to produce compounds that influence beef flavor and react with components of lean tissue to give distinct flavor compounds (Mottram, & Edwards, 1983).

Correlations between 14:1, 16:1, 18:0, 18:1, 18:2, 18:3, and desirable beef flavor have been reported (Melton et al., 1982). It is important to remember the species flavor depends more on unsaturated aldehydes, but fatty acids, ketones, and saturated aldehydes
all play a role in beef flavor especially since many of the aldehydes are derived from pathways with fatty acids (Melton et al., 1982).

**Fat content**

Fat content has been shown to affect palatability traits including flavor. As intramuscular fat increases, the fat flavor increases which is preferred by most consumers (Miller, Moeller, Goodwin, Lorenzen, & Savell, 2000). The minimal level of intramuscular fat for consumer acceptance and preference, described as slightly intense fat flavor, is approximately 3% (Miller, 2001). However, levels of fat above 7.3% in meat have a negative effect on perception of flavor and acceptability (Miller, 2001). Francis, Romans, and Norton (1977) also found this bell-shaped curve for marbling’s effect on flavor. Loin steaks had a linear decrease in flavor desirability as quality grade went down from USDA Prime through USDA Cutter (Smith et al., 1983). Flavor desirability ratings in top round steak were less affected by grade and especially marbling score.

The difference in flavor of beef and beef cuts is heavily influenced by fat content. The content of total lipids, polyunsaturated fatty acids, monounsaturated fatty acids, and saturated fatty acids for numerous beef cuts with varying levels of trim, cooked and raw, and USDA Prime, Choice, and Select can be found in the USDA Nutrient Standard Database.

**Triacylglycerides and Phospholipids**

To determine the effect fats from triacylglycerides (TAG) and phospholipids (PPL) have on the aroma of cooked beef, a control and three solutions were prepared to remove either the TAG, PPL, or both portions of fat from the meat samples (Mottram et
Using triangle tests, panelists were unable to distinguish the difference between the control and when TAG were removed from the sample, and described both aromas as meaty. The samples with the PPL removed and the TAG+PPL were described as roasted/toasted. Gas chromatograph results determined alcohol volatiles were unchanged when TAG were removed with a main component being 1-octen-3-ol. In the PPL and TAG+PPL removed samples the level of 1-octen-3-ol was 200 times less, but there was a 4-5 fold increase in benzaldehyde. Pentylfuran followed the same trend as the alcohols in the three treatments. Interestingly, there was a slight increase in pyrazines, responsible for the nutty, roasty aroma, in the TAG removed samples and a much more significant increase in the PPL and TAG+PPL removed samples. This large increase, once the meat was defatted, suggests lipid in meat may inhibit the formation of pyrazines. Benzaldehyde increased in all the treatments which was unexpected since it was believed that benzaldehyde only came from the thermal oxidation of linoleic acid. The increase revealed there is another pathway in which benzaldehyde can be formed in meat (Mottram et al., 1983).

**Fatty Acids**

Refer to the diet section in live animal factors for discussions on the effect of feedstuffs on fatty acids affected by feedstuffs. In general, fatty acid composition of beef is very hard to manipulate because of the biohydrogenation process in the rumen and enzymes and bacteria in the intestines. Biohydrogenation converts feedstuffs to mainly stearic acid, the main saturated fatty acid found in meat. The rib and loin primals have the most fatty acids in the lean portion of the retail beef cuts. The lipid portion in the lean
is typically slightly higher in monounsaturated fatty acids than saturated fatty acids (Miller, 2001).

Castration plays a role in the development of fatty acids in an animal. Meat from intact beef animals on the same feeding and management regime and adjusted for intramuscular fat had higher levels of C17:0, C18:1 trans, C18:2 n-6, PUFA:saturated fatty acids, n-6: n-3, C18:2n-6: C20:4n-6 and lower values of C16:0 and C18:1 cis-9 than meat from castrated animals (Monteiro, Santos-Silva, Bessa, Navas, & Lemos, 2006). Interestingly, several of these fatty acids have been found to be higher in animals with more off-flavors (Camfield, et al., 1997; Jenschke, Hodgen, Calkins, 2006).

To determine the volatile compounds that are derived from linoleic acid and methyl linoleate, Ullrich & Grosch (1987) used gas chromatography to derive a D-value (the highest dilution at which a substance is still smelled) to reveal the most intense flavor compound. With both lipids, hexanal, 2-octenal, and 2-nonenal had the highest D-values. The fourth highest compound for linoleic acid was 1-octen-3-ol while 1-octen-3-one was for methyl linoleate. After 24 h of linoleic autoxidation, 2-nonenal was the most potent volatile, with hexanal the most potent volatile after 48 h, and hexanal and 2-octenal the most potent volatiles after 72 h. The authors also stated pentane is a better indicator of lipid peroxidation because it has a shorter induction period than other volatile compounds even though its D-value is not as high.

Arachidonic acid, a long chain omega-6 polyunsaturated fatty acid, was originally found to be autoxidized in meat into hexanal, methyl 5-oxopentanoate, pentane, and 2,4-decadienal volatile compounds (Artz, Perkins, & Salvador-Henson, 1993). However, the most intense aroma compound from the oxidation of arachidonic acid is trans-4,5-epoxy-
(E)-2-decenal followed by 1-octen-3-one, 2,4-decadienal, 2,4,7-tridecatrienal, and hexanal (Blank, Lin, Vera, Welti, & Fay, 2001).

There is an increasing desire to increase the PUFA in the lean portion of meat. However, when levels become too high, off-flavors can develop, especially during cooking (Elmore et al. 2002). These off-flavors develop in four ways: 1) The breakdown products of $n$-3 acids have shorter chain lengths so they are more volatile with lower flavor thresholds than the $n$-6 and $n$-9 acids. Therefore, they will also be in greater concentrations than the more unsaturated longer chain fatty acids. 2) The $n$-3 breakdown products have a higher proportion of double bonds to chain length so they are more reactive than $n$-6 acids. These products will interact with Maillard products to reduce the number of sulfur-based meat aroma compounds. 3) The interaction between the $n$-3 and Maillard products will create their own aroma. 4) The $n$-3 PUFA are more readily oxidized than $n$-6 or $n$-9 so they could initiate the radical oxidation of more saturated fatty acids and increase the breakdown of $n$-6 and $n$-9 acids to alter the cooked meat aroma profile.

**Other**

*Thiamin*

This nonvolatile vitamin has been shown to produce odor-causing compounds in meat. Thiamin produces intermediates like 5-hydroxy-3-mercapto-2-pentanone when heated. This intermediate then reacts to form sulfur-containing compounds that give meat a cooked and roasted aroma. These intermediates can also interact with other Maillard reaction intermediates and end products like 4-methyl-5-(2-hydroxyethyl)thiazole, ammonia, hydrogen sulfide, and formaldehyde to form furans,
thiophenes and 2-methyl-3-furanthiol, a major meaty aroma compound (Jhoo et al., 2002). Thiamin in the presence of cysteine increases the formation of 2-methyl-3-furanthiol.

*Sulfur*

Pentose sugars, especially inosine-5’-monophosphate which accumulates post-harvest, and cysteine are the precursors to the Maillard reaction. The intermediate in the reaction is 4-hydroxy-5-methyl-3(2H)-furanone and dicarbonyls such as butanedione and pentanedione. These intermediates interact with disulfides or cysteines for end products. Therefore, it has been suggested the amount of inosine-5’-monophosphate at the time of cooking is a factor influencing intensity of meaty flavor (Mottram & Madruga, 1994).

When thiol groups sit in the 3-position of furans and thiophenes, characteristic meat-like aromas can also be produced. These furans and thiophenes oxidize to form disulfides with low odor thresholds.

In meat, sulfur can be produced from cysteine, glutathione, proteins, and thiamine. Once thermal processes (Maillard reaction) have begun, these sources of sulfur can interact with other compounds in meat to form the volatile sulfur compounds (Tai, & Ho, 1998). Oxygen and pH contribute to glutathione, needing less activation energy than cysteine to produce hydrogen sulfide, which explains why its contribution to meat aroma is so significant (Tai et al., 1998). The free radicals from lipid oxidation can degrade sulfur-containing compounds in meat which increases the levels of aliphatic and cyclic sulfur compounds (Drumm, & Spanier, 1991).

Sulfur compounds at low concentrations possess a pleasant meaty aroma, but their odor is objectionable when the concentration is high (Shahidi, 1989).
Many compounds contributing to beef flavor are said to be water-soluble. As pH increases in meat, the proteins have increased water binding properties. During cooking fewer water soluble proteins are lost from high pH meat since there is less cooking loss (Miller, 2001). Dark, firm and dry (DFD) meat is said to have a musty/moldy, very high beef flavor intensity, cowy/grassy, or bloody/serumy aromatic flavors. High levels of sodium and phosphate can lead to some of the same flavor perceptions that are in DFD meat. While carcasses with lower than average pH are not common, the meat from these animals is usually blander. Lower pH levels can also be attained through the use of acidic ingredients (Miller, 2001).

Degree of Doneness

Crocker (1948) described raw meat flavor as weak, sweet, salty, and blood-like and through cooking the desirable meat flavors develop. Bowers, Craig, Krophp, & Tucker (1987) cooked strip steaks to seven different endpoint temperatures, 55-85°C. Shear force, compression, and pH were not affected by endpoint temperature, but all panel ratings for flavors and juiciness were affected. Temperature changes between 60-65°C and 80-85°C were determined to cause the biggest changes to flavor. Mouth-filling blend increased significantly at these two temperatures while bloody/serumy, metallic, and sourness decreased. Protein denaturation occurs at approximately those temperatures which may explain the decrease in bloody/serumy notes. Juiciness ratings had a linear decline over the temperatures, as expected.

Beef roasts were cooked to four different internal temperatures to evaluate the flavor profile created (Belk, Miller, Evans, Liu, & Acuff, 1993). Increased temperatures
increased the cooked beef/brothy, cowy/grainy, and cardboardy aromas as well as the liver-like aromatic. Cooked beef fat aroma was not affected by endpoint temperature, but serumy/bloody, painty, and soured aromatics were always higher at lower temperatures. Additionally, metallic and astringent mouthfeel and sour and bitter tastes were at a more intense level in the roasts cooked to a lower degree of doneness.

Six muscles from the chuck and round were evaluated for flavor characteristics at two endpoint temperatures of 66°C and 77°C (Calkins, 2002). The steaks cooked to the lower temperature were perceived as juicier, more intense in flavor, and higher in flavor preference. The same trend was seen in Streff, Wulf, & Maddock (2003) at endpoint temperatures of 63°C and 79°C. In this study browned flavors were not perceived to be different at the higher temperature, but charred and oxidize were. Acidic, sour, and metallic notes were lower at the higher temperature as well. The *M. infraspinatus* had the lowest off-flavor and highest flavor intensity, but was the only steak affected (negatively) by the increased cooking temperature. Streff et al. (2003) found the opposite result with the *M. infraspinatus* having the highest incidence of off-flavors, although only three of the seven muscles were the same. The *M. psoas major* and *M. infraspinatus* had the highest incidence of liver-like flavors as well (Streff et al., 2003). The *M. tensor fasciae latae* was very similar to the *M. infraspinatus* in terms of flavor characteristics (Calkins 2002). While it is recommended that trained panels not be asked flavor preference, the *M. teres major* received the lowest flavor preference scores in addition to the highest off-flavor intensity.

As degree of doneness increases, serumy/bloody, metallic, sour, and bitter notes decrease while liver-like and cooked beef brothy can increase (Miller, 2001). Other
studies have shown a close relationship (correlation) with metallic and liver-like flavors so degree of doneness may not be the only contributing factor for these flavors (Insausti, Beriain, Gorraiz, & Purroy, 2002).

Other contributing factors

Numerous studies have shown an impact on flavor from various sources beside the ones contained in this section. Some of these topics include breed, irradiation, enhancement, marination, taste receptor blockers, and seasonings.

Compounds

Sulfurous and carbonyl compounds seem to be the predominant contributor to meat flavor (Shahidi, 1989). Gasser & Grosch (1988) established 2-methyl-3-furanthiol and bis(2-methyl-3-furyl) disulfide contributed to the desirable aroma of beef when isolated from other possible compounds contributing to meat aroma. Bis(2-methyl-3-furyl) has an odor threshold of 0.02 ng/g in water while methylating 2-methyl-3-furanthiol increases the odor threshold. This suggests 2-methyl-3-(methylthio)furan is only a minor contributor to the flavor of meat as suggested by MacLeod (1986). Gasser & Grosch (1988) also established flavor dilution factors (FD; aroma extracts are stepwise diluted until sniffers cannot detect odorants with the highest level reported as flavor dilution) to evaluate the contribution of the compound to the overall beef flavor. Forty compounds were determined in cooked beef to have a FD larger than four, and 17 compounds had a FD larger than 64 which where stated to be major contributors to cooked flavor. Farmer and Patterson (1991) isolated five compounds that were found to be desirable to cooked beef flavor: bis(2-methyl-3-furyl) disulfide, 2-furfuryl 2-methyl-3-
furyl disulfide, bis(2-furfuryl) disulfide, dimethylfuryl 2-methyl-3-furyl disulfide, 2-methyl-3-furyl 2-methyl-3-thienyl disulfide.

A Strecker aldehyde, methional, is a low threshold (0.2 μg/g in water) sulfur compound that is described as ‘pleasant, warm-meat, or soup-like’ (Gasser et al., 1999). The roasty note in beef is in part produced by 2-acetyl-1-pyrroline and 2-acetylthiazole. Many compounds that contribute to meat smell and flavor are lipid breakdown products. Fatty acids such as linoleic and arachidonic acid start to autoxidize to 9-hydroperoxide and 11-hydroperoxide, respectively, which can form 2,4-decadienal, 2-nonenal, 1-octen-3-one, 2,4-nonadienal, and 2-octenal through β-scission with 2-nonenal and 2,4-decadienal having as high of FD values as the sulfur compounds contributing to meaty flavor.

Through oxidation of β-carotene, a very intense aromatic β-ionone is formed (Sanderson, Co, & Gonzalez, 1971) which likely comes from animal feed (Gasser et al., 1988). One compound responsible for the tallowy and/or beef-like smell is 12-methyltridecanal (Guth, & Grosch, 1993), a compound derived from plasmalogens, glycerophospholipids with a long chain fatty aldehyde linked to the sn-1 position by a vinyl ester bond (Guth et al., 1993; Dannenberger, Lorenz, Neumberg, Scollan, Ender, & Neumberg, 2006). The 12-methyltridecanal’s odor threshold is 0.1 μg/kg in water and is found in higher amounts in lean meat from beef than other species (Guth et al., 1993). Increasing the amount of forage in the diet of an animal increases 12-methyltridecanal in the phospholipids of muscles as well as n-octadecanal, another plasmalogen. Octadecenal, a monounsaturated plasmalogen with a low odor threshold, was reduced
when forages were fed instead of concentrates, but $n$-hexadecanal concentration was not affected by diet (Dannenberger et al., 2006).

Hexanal and 2,4-decadienal contribute positively to beef flavor, but at higher concentrations it is possible to produce undesirable flavors (Melton, 1983). This is probably because these two compounds are produced in the greatest amounts during oxidation of 18:2 during heating as well as overshadowing compounds that also help produce typical beef flavors. Others have found that hexanal is the most prominent volatile compound in cooked meat with the amount being directly proportional to TBARS and inversely proportional to flavor acceptability (Shahidi & Pegg, 1994; Ullrich et al., 1987).

Numerous factors influence the flavor of meat. It is this complex relationship between production, processing, and cooking that develops the compounds that create the unique matrix of ‘meaty’ flavor in beef.


References


Melton, S. L., Black, D., Davis, & Backus. (1982). Flavor and selected chemical characteristics of ground beef from steers backgrounded on pasture and fed corn up to 140 days. *Journal of Food Science, 47*(3), 699-704.


Figure 1. Compounds identified from cooked beef aromas and reported to possess meaty odor (Shahidi, 1998).
Figure 2. General scheme of the Maillard reaction occurring in food (Hodge 1953).
Figure 3. Initial steps of the Maillard reaction to produce the Amadori rearrangement end products.
Figure 4. Some thiols, sulfides and disulfides from meat which contribute to meaty aroma (Mottram et al., 1994).
Table 1. Ranking of muscles\(^a\) for flavor intensity from different studies

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\(^a\) AD = M. adductor; BB = M. biceps brachii; BF = M. biceps femoris; BT = M. brachiocephalicus; CP = M. complexus; DI = Diaphragm; GM = M. gluteus medius; GR = M. gracilis; IC = intercostal muscles; IF = M. infraspinatus; IP = M. ilio psoas; LD = M. longissimus dorsi; LT = M. latissimus dorsi; MD = M. multifidus dorsi; OA = M. obliquus abdominis internus; PM = M. psoas major; PP = M. pectoralis profundi; QF = M. quadriceps femoris; RA = M. rectus abdominis; RB = M. rhomboideus; RF = M. rectus femoris; SD = M. spinalis dorsi; SF = M. superficial pectoral; SL = M. splenius; SM = M. semimembranosus; SP = M. supraspinatus; SS = M. subscapularis; ST = M. semitendinosus; SV = M. serratus ventralis; TB = M. triceps brachii; TF = M. tensor faciae latae; TM = M. teres major; TR = M. trapezius; VI = M. vastus intermedius; VL = M. vastus lateralis; VM = M. vastus medialis

\(^b\) Samples are ordered from the most intense beef flavor intensity to the least (bland)

\(^c\) Means within column without common superscript differ

1 Shackelford et al., 1995; 2 Paterson et al., 1986; 3 Jeremiah et al., 2003; 4 Carmack et al., 1995; 5 Jeremiah et al., 1985; 6 Molina et al., 2005; 7 Rhee et al., 2004; 8 Wheeler et al., 2000; 9 Brickler, 2000, dry cookery; 10 Brickler, 2000, wet cookery; 11 Wulf et al., 2000
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a AD = M. adductor; BB = M. biceps brachii; BF = M. biceps femoris; BT = M. brachiocephalicus; CP = M. complexus; GM = M. gluteus medius; GR = M. gracillis; IF = M. infraspinatus; LD = M. longissimus dorsi; LT = M. latissimus dorsi; MD = M. multifidus dorsi; PM = M. psoas major; PP = M. pectoralis profundus; QF = M. quadriceps femoris; RB = M. rhomboideus; RF = M. rectus femoris; SF = M. superficial pectoral; SM = M. semimembranosus; SP = M. supraspinatus; SS = M. subscapularis; ST = M. semitendinosus; SV = M. serratus ventralis; TB = M. triceps brachii; VI = M. vastus intermedius; VL = M. vastus lateralis; VM = M. vastus medialis

b Samples are ordered from the lowest off-flavor score to the highest off-flavor score

c-g Means within column without common superscript differ

1 Shackelford et al., 1995; 2 Rhee et al., 2004; 3 Brickler, 2000, dry cookery; 4 Brickler, 2000, wet cookery
Review of Literature

Part III. Methods to Isolate Volatile Compounds

Introduction

The scientific community has started looking at the molecular level and smaller to increase the knowledge base on flavor. With this interest, new and/or improved technology has been introduced. Flavor chemistry has looked to other fields to gain insight into technology that could be useful in the food industry. There are numerous applications for isolating and identifying flavor compounds. Determining flavor profiles, developing new flavors, and identifying compound(s) causing pleasant or unpleasant odors are reasons for these efforts. The following review describes three of the main types of methods for isolating flavor compounds for identification with analytical equipment.

Static Headspace

Obtaining volatiles from a solid sample using a direct analysis is probably most commonly done with static headspace as it is the simplest. Typically, a liquid or gas sample is placed in a closed container, and the volatile compounds in the sample are allowed time to equilibrate between the sample and the headspace above the sample. An aliquot is taken from the headspace and injected into a gas chromatography (GC) column (Figure 1). This method is significantly affected by the sample matrix, especially when volatiles are soluble in the matrix (Butler et al., 2003).
Solid Phase Micro Extraction

To view an animation of the solid phase microextraction (SPME) sample absorption process animation please visit Sigma’s website:

Solid phase microextraction is a quick, solventless assay to separate particulates in a sample. There are several benefits to this method including elimination of steps (reduction of sample preparation time) that can change the properties of volatile compounds and elimination of hazardous solvents. As a result, early eluting compounds are not masked by the solvent peak. This extraction process is relatively inexpensive in comparison to headspace or purge and trap, and is reusable since the fiber can be used up to 50 times (www.sigma.com). In the early 1990s Dr. Janusz Pawliszyn of the University of Waterloo in Ontario, Canada introduced the method (US patent number 5691206; Pawliszyn, 1997) in which a specially-coated, silica fiber was placed on a syringe-like device to extract desired sample volatiles that could be analyzed with GC (Arthur, & Pawliszyn, 1990). Further automation and development of the technique has allowed for a wide range of applications with GC, liquid chromatography, mass spectrometry (MS), supercritical fluid chromatography, and capillary electrophoresis.

This technique can be used in liquid samples by absorption or with solid samples by adsorption. In general, the volatiles from samples are exposed to a coated, fused silica fiber for 2 to 30 min. A gas or liquid sample or the headspace above a liquid or solid sample can be tested. The fiber is retracted, and the needle is removed from the sample. The needle can be inserted into the injection port of a GC or inserted into the SPME/High Performance Liquid Chromatography (HPLC) interface desorption chamber for analysis.
Higher boiling compounds can be extracted at higher resolutions by increasing the time the volatiles are in contact with the fiber (Rouseff, & Payne, 2000).

Two common types of SPME are utilized: direct and headspace. Direct is best used for medium to low volatility, high to medium polarity in water, and a gas or liquid sample. Headspace (discussed in detail later) is more effective with high to medium compound volatility, low to medium polarity, and a liquid or solid matrix (Garner, & Smith, 2004b).

To improve performance of SPME, the volatility of an analyte can be increased by heating the sample. However, the sample must be equilibrated at the desired temperature before the fiber is inserted into the sample.

*Fiber*

Adsorption type filters are better for extracting analytes present at low concentration levels and provide lower minimum detection levels. Absorption fiber coatings are used for semi-volatile compounds, and because these fibers use partitioning, they have greater capacity and linear concentration ranges than adsorption fibers (Rouseff, & Payne, 2000).

Fibers are 1-2 cm long with numerous stationary phase film thickness (7 μm-100 μm) which allows collection of different volumes of analytes. There are seven polymer phases of the fibers available through Supleco (member of the Sigma-Aldrich group, Bellefonte, PA), the only provider of commercial fibers. These phases are polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA), carbowax/divinylbenzene (CW/DVB), polydimethylsiloxane/carboxen (PDMS/CAR), polydimethylsiloxane/divinylbenzene/carboxen 1006
(PDMS/DVB/CAR), and carbowax/templated resin (CW/TPR) (Garner, & Smith, 2004a).

The key to the SPME method lies in the fiber. Unique fibers are available for specific uses. Shirey (2000) investigated the ability of different fibers for specific applications. He determined adsorbent coatings should be used for trace concentration levels while PDMS allows investigation of sub-percent levels which means PDMS/CAR fibers extract most volatile compounds, PDMS/DVB/CAR is best for semi-volatile compounds, and PDMS/DVB should be used for amines.

**Headspace SPME**

Organic compounds that might interfere with the analysis may be minimized by sampling in the headspace above the sample (Rouseff, & Payne, 2000). This modification to SPME enables analysis of solid samples and shortens the extraction time. The detection levels are not compromised compared to direct SPME (Figure 2), and one can detect parts per trillion when an ion trap mass spectrometer is used as a detector (Zhang, & Pawliszyn, 1993).

In fact, a compound with Henry’s constant above 90 atm-cm³/mol can be isolated at ambient temperatures. These compounds could include three-ring polynuclear aromatic hydrocarbons and more volatile particles in addition to the less volatile compounds. To obtain the less volatile compounds in a shorter equilibrium time, the aqueous phase and headspace require agitation, the volume of headspace should be reduced, and/or the temperature should be increased (Zhang, & Pawliszyn, 1993). The use of headspace SPME for flavor volatiles was introduced in 1996 by Steffen, & Pawliszyn using orange juice. The headspace SPME was used to demonstrate TBARS
and hexanal correlations for oxidation of pork from pigs on different diets and refrigerated storage days (Fernando, Berg, & Grun, 2003) as well as other meat studies.

Other SPME Modifications

As technology continues to increase, methods for more specialized compounds will be developed as adaptations to the SPME method. Matrix assisted laser desorption ionization (MALDI), electrospray ionization (ESI), and nanospray can enable analysis of polar, non-volatile compounds like peptides and proteins when coupled with MS. The MALDI system (Figure 3a-b) uses a laser for energy to singly charge protonated molecules with high molecular weight, non-volatile, and thermally reactive (Tong, Sze, Thomson, Nacson, Pawliszyn, 2002) while the ESI/nanospray system (Figure 4) has a period of desorption of particles in a small volume of solvent followed by direct ionization (Walles, Tong, Thomson, Nacson, & Pawliszyn, 2003). By adding SPME to these ionization techniques, all the components are in one fiber for simplification of biomolecular analysis.

Purge and Trap GC/MS

Purge and trap is a dynamic headspace analysis technique also called direct thermal desorption. A major benefit to this analysis is the reduction of the sample matrix effect. In this system, samples with volatile organic compounds are placed in a purge container and flushed with an inert gas at a constant flow rate for a specific time (usually 30-70 mL/min for 10-15 min) to remove the volatiles from the sample and push the molecules through a heated valve. This ‘wet’ purge removes the majority of the water, and a dry purge can follow if further water removal is necessary. Volatile compounds are concentrated into the absorbent trap. When the purging is complete, the trap is heated
quickly and backflushed with carrier gas to desorb (typically 2-4 min at 180-250°C with
a flow rate of 10-80 mL/min) the compounds in a narrow band for analysis in the GC.
Polar and nonpolar molecules should be retained with minimal water and methanol.

Purge Temperatures

In raw turkey thigh meat, major volatile components could change with the purge
temperature, but the total number of volatiles did not increase until the samples were
purged at 80°C (Ahn, Jo, & Olson, 1999). The greatest change in composition was when
the temperature was changed to 80°C from 60°C. However, the changes in raw meat
were not as significant as effects of purge temperature on the volatiles in cooked turkey
thigh meat. Hexanal increased the most, but all compounds except alkanes increased.
The major reason for the high volatile production in the cooked meat samples was lipid
oxidation since cooked meat is more susceptible to lipid oxidation than raw meat (Ahn,
Ajuyah, Wolfe, & Sim, 1993; Ahn, Wolfe, Sim, & Kim, 1992). The final
recommendation for purge temperature was 40-50°C for raw and cooked turkey meat
(Ahn et al., 1999). Storage time in the autosampler before purging was also found to be
as important as purge temperature in the study. Ahn et al. (1999) recommended samples
be in the autosampler less than 3 h unless the samples were purged with helium or
nitrogen.

Trap Selection

Numerous traps are available, with various absorbent material. The weakest
absorbent material is placed at the inlet followed by successively stronger absorbents.
The lower volatility samples are absorbed in the first layer, while the higher volatility
compounds are absorbed in the stronger absorbents. A summary of common absorbents follows (Butler et al., 2003).

-Tenax®: has a surface area of 50 m²/g, is hydrophobic, and traps non-polar compounds. Disadvantages to this absorbent are extremely volatile compounds and alcohols are not retained, limited thermal stability, particles can melt together and adhere to trap, and a loss in response to brominated molecules occurs as the adsorbent degrades.

-Silica gel: has a surface area of 200-800 m²/g and is ideal for trapping polar and highly volatile compounds that are gases at room temperature. However, it is very hydrophilic.

-Coconut charcoal: With a surface area of 900 m²/g, it is commonly used after silica to trap very volatile compounds. While charcoal is hydrophobic, it will trap carbon dioxide and interfere with early eluting compounds.

-Carbon molecular sieves: This absorbent has a surface area of 800 m²/g, can be an alternative absorbent for silica gel and/or charcoal, and is excellent for trapping highly volatile compounds. Additionally, the carbon molecular sieve is hydrophobic with great thermal stability (Butler et al., 2003).

Most traps are specific to the brand of purge and trap system being used. Therefore, this review of traps will focus on the traps available for use in the system our laboratory utilizes (O-I-Analytical Model 4560 Purge & Trap Sample Concentrator, College Station, TX). The original trap for purge and trap analysis had a 1 cm section of sorbent on a support, with 7 cm each of Tenax®, silica gel, and activated charcoal. The Tenax® captures compounds with boiling points higher than 35°C, the silica gel captures
compounds below 35°C (except compounds with volatility like dichlorodifluoromethane), and the activated charcoal traps the highly volatile compounds like dichlorodifluoromethane. Because the original sorbent caused excessive bleed and artifact compounds, the replacement had 8 cm sections of the Tenax®, silica gel, and activated carbon. With standard conditions of 11 min purge at 40 mL/min purge flow, very low boiling compounds will not be captured (O-I-Analytical Application Note).

Today, most applications require Trap 7-10. A 24 cm Tenax® trap (Trap 7) is recommended when compounds of interest have boiling points above 35°C which is useful with samples in solvents like methanol because the solvents can be removed from the trap with a dry purge. If highly volatile compounds like dichlorodifluoromethane are not of interest, a trap (Trap 8) with 16 cm Tenax® and 8 cm of silica can be used for better performance for the desired compounds. Trap 9 (8 cm Tenax®, 8 cm silica gel, and 8 cm activated charcoal) is recommended for general use. To reduce background noise due to carbon dioxide with equal trapping and desorb performance, the Trap 10 with 8 cm Tenax®, 8 cm silica gel, and 8 cm carbonized molecular sieve is recommended. Trap 10 also allows the system to be used with a mass spectrometer. Trap 11-12 are VOCARB® rather than Tenax® when the purge and trap system is being used with an ion trap mass spectrometer. The VOCARB® traps are the most effective traps available because of the ability to retain very volatile compounds with minimal bleed, activity, or breakdown as well as trapping higher boiling point compounds without the need for moisture control systems. O-I-Analytical has patented a MicroTrap to allow splitless injections to the analytical column which would increase sensitivity.
Other methods to isolate, separate, and identify compounds are available. Because of the nature of this research, they will not be discussed but acknowledgement needs to be given to some of the analytical methods that have increased the knowledge and identification of compounds, their corresponding flavors, and the effect on the food system. Gas chromatography-olfactory (GC-O; GC with a human olfactory port to smell compounds as they come off) has many uses including using human sense to rate intensity and give a flavor to a particular compound(s). While the electronic nose and mouth are still in their developmental infancy, continued efforts to improve the technology will make testing much more convenient and portable.

Comparison Between Methods

Pfannkoch & Whitecavage (2000) compared three direct analyses of volatiles from solid matrices conducted under the same conditions. Static headspace was the least sensitive, but gave adequate information if substantial sample size was available and when there was high water content in the matrix. Solid phase microextraction with the 100 μm polydimethylsiloxane fiber was 10-50 times more sensitive than static headspace, and was useful with high moisture samples. Direct thermal extraction (purge and trap) was 50-100 times more sensitivity than SPME and 500-5,000 times more sensitive than static headspace in addition to only needing a small sample size. Two problems arose from direct thermal extraction. With the small sample size, variable results were obtained because samples were not homogeneous. This analysis also needs an inlet liner to eliminate water interferences with higher moisture samples.
References


Pfannkoch, E., & Whitecavage, J. (2000). Comparison of sensitivity of static headspace GC, solid phase microextraction, and direct thermal extraction for analysis of


**G=gas phase (headspace)**
The gas phase, commonly referred to as the headspace, is above the sample phase.

**S=sample phase**
The sample phase contains the compound(s) of interest, usually in the form of a liquid or solid in combination with a dilution solvent or matrix modifier.

Once the sample phase is introduced into the vial and the vial is sealed, molecules of the volatile component(s) diffuse into the gas phase until the headspace reaches a state of equilibrium, as depicted by the arrows. An aliquot is then taken from the headspace.

Figure 1. Static Headspace. Volatile analyte in equilibrium between the gas and sample phases. (Butler et al., 2003).
Figure 2. Direct SPME versus Headspace SPME (recreated from http://www.science.uwaterloo.ca/chemistry/pawliszyn/Research/SPME/spme5.gif)
Figure 3a. Schematic diagram of SPME/MALDI-ion mobility spectrometer (IMS) system: (1) laser source, (2) focusing lens, (3) photodiode, (4) fiber holder, (5) SPME/MALDI fiber, (6) IMS, (7) oscilloscope, (8) glass tube, (9) fixing septum, $V_0$ and $V$, designed and effective high voltage in drift tube, respectively; $L_o$ drift tube length; $L$, ion drift length; $t_d$, ion drift time.

Figure 3b. Schematic diagram of SPME/MALDI quadrapole (Qq) time-of-flight (TOF) tandem mass spectrometer (MS) system (1) laser source, (2) focusing lens, (3) fiber holder (4) SPME/MALDI fiber, (5) QqMS, (6) TOF MS. (Tong, Sze, Thomson, Nacson, Pawliszyn, 2002)
1) Extraction

2) Desorption

3) Analysis

Body Fluid

Analyte

Wash

Coating

Desorption-Solvent

Nanospray-Tip

SPME-Device

Counter-Electrode-Cap

Distance 0.5-2 mm

to ground

Figure 4. SPME/Nanospray Schematic (Walles et al., 2003).
The purge and trap concentrator in “purge” mode. The 6-port valve allows carrier gas to bubble through the aqueous sample, transferring volatiles to the absorbent material.

The purge and trap concentrator in “desorb” mode. VOCs concentrated on the trap are desorbed to the chromatograph for separation, identification and quantification.

Figure 5. The modes of action by the purge and trap system (Butler et al., 2003).
Materials And Methods

STUDY 1

The Influence of Cooking Rate and Holding Time on Beef Chuck and Round Flavor

Sample Collection

Shoulder clods (IMPS #114; NAMP 1997) and knuckles (IMPS #167; NAMP 1997) from 12 (n=6 USDA Choice; n=6 USDA Select) A-maturity beef carcasses were collected from the federally inspected Cargill Meat Solutions plant in Schuyler, NE on June 2, 2004. Identity of the animal was maintained for both the knuckles and clods by tagging the respective location prior to fabrication at the plant. Carcass data were only obtained on six of the carcasses because the other six tagged carcasses were rail headed to fabrication. The muscles from two carcasses (1 = Choice and 1 = Select) were not used in this study. The primals were brought to the Loeffel Meat Lab at the University of Nebraska and allowed to age in vacuum bags in a 1°C cooler for 7 d post slaughter.

Muscle Preparation

Seven muscles (M. infraspinatus - INF, flat iron; M. teres major- TER, shoulder tender; M. triceps brachii- TRI, clod heart; M. rectus femoris- REC, knuckle center; M. vastus lateralis- VAL, knuckle side; M. vastus medialis- VAM, knuckle bottom; and the M. vastus intermedius- VAI, knuckle soft) located in the clod and knuckle from ten animals (5=Choice and 5=Select) were separated and trimmed of external fat after aging 7 d post-harvest. The term value cut refers to the beef muscles, mainly from the chuck and the round, that are being considered for utilization other than ground beef or roasts. A 100 g portion of the proximal end of each muscle was removed and minced for chemical analysis. The thick band of connective tissue running through the center of the
INF was removed. The TRI, REC, and VAL were cut into 2.54 cm steaks. The top and bottom portions of the INF were cut in half to make four steaks. The TER, VAM, and VAI were cut in half. Steaks were wrapped in freezer paper and frozen (-16°C) until sensory evaluation was conducted.

**Panelist Training**

Taste panelists were recruited through a classified ad in the *Lincoln Journal Star* that ran from April 18-21 and April 25, 2004. Seven individuals responding to the advertisement were retained to train for sensory evaluation of beef muscle. Four additional individuals from the Animal Science department staff and graduate students were recruited for training. Screening of the individuals prior to training was performed to determine if they could detect average threshold levels of sour, sweet, bitter, and salty according to Jellinek (1985). Two days of screening were done using triangle tests and paired comparison of 1% of the following solutions in water to ensure panelists could detect differences between the tastes.

- **Sweet** - Sucrose
- **Sour** - REALemon™ Juice
- **Bitter** - Coffee Extract (two cups Folgers coffee grounds placed in cheese cloth with two cups of cold double, distilled water run through it)
- **Salt** - Iodized Salt

Panelists identified at least 60% correctly from the triangle test and 75% correctly from the paired comparison (Meilgaard, Civille, & Carr, 1991).

Training for sensory evaluation of meat was completed according to the AMSA *Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness*
Measurements of Fresh Meat (1995) on 13 dates between May 11, 2004 and July 13, 2004. Panelists received $7.50/session for training. Panelists were asked to taste a sample, rate the sample for tenderness, juiciness, connective tissue and off-flavor intensity, identify specific off-flavors, and then discuss the results to understand why a sample obtained a specific score. Sirloin steaks (multiple muscles), strip steaks (M. longissimus lumborum), eye of round steaks (M. semitendinosus), round steaks, and tenderloins (M. psoas major) with inherent connective tissue and tenderness difference were used. Some days all five types of steaks were served; other days only 1-2 steak types were prepared for training. Strip steaks cooked to varying degrees of doneness (65°C, 70°C, and 80°C) with shear force ranging from 2.37-6.22 kg were used to train for tenderness and juiciness. Eye of round steaks were also cooked to the three degrees of doneness to evaluate juiciness. Each day one sample, with two different, three-digit random numbers, was evaluated twice so panelists could be evaluated for consistency.

Off-flavor descriptors were derived from the panelists with the active assistance of the trainer. Several panelists from within the department had previous training so their suggestions for terms were generally the suggested term for the off-flavor such as warmed-over flavor and metallic. Most of the training for off-flavors was done using strip steaks and value cuts aged different amount of days and varying the degree of doneness. The REC, INF and VAL were the value cuts used to help panelists identify metallic, fatty, and sour off-flavors. The term warmed over/oxidized was used for the flavor derived from meat samples left in the refrigerator for an additional day and microwaved for either a 1.5 min interval or a four min interval, and then grilling on an electric broiler (FSR200, Farberware Inc., Prospect, IL) for color. Additional metallic
off-flavor training was conducted by needle-injecting meat with varying levels of potassium chloride. Salt flavors were identified by needle-injecting 0.5% (four on off-flavor scale), 1.0% (three on off-flavor scale), or 2.5% (1 on off-flavor scale) (w/w) salt in water solution into the meat samples. Anchors for liver-like off-flavor were established. Samples were made using 80% lean ground beef and in a particular percentage of course ground liver. The liver and ground beef were reground to gain a more homogenous mixture. The liver and ground beef patties were cooked to an internal temperature of 70°C on the Vulcan commercial gas grill (VCCV 36-1; Vulcan Hart Corp., Louisville, KY). Samples that were 25%, 30%, or 50% liver were a one in off-flavor intensity while those with 10%, 1%, and 0.5% added liver were a two, six, and seven, respectively, in off-flavor intensity.

**Sensory Analysis**

Panels were run from July 14 - Aug 19, 2004 with sessions either mid morning or mid afternoon so at least an hour had passed since panelists had consumed soft drinks, coffee, or food. Panelists received $10 for panels and a small piece of candy after the session. Four steaks from one USDA Choice and four steaks from one USDA Select muscle type were randomly ordered and served one sample at a time during every taste panel session. In one session the panelists received four TER and four VAI samples. There was a 5 min break between serving the second muscle type. Serving order of muscles was randomized as was steak location (second through fifth steaks counted from the anterior end of the muscle) for each cooking and holding treatment. All four locations were used for each treatment combination, however.

Steaks were thawed in a 3°C cooler 24 h prior to cooking for sensory evaluation.
One steak from each muscle was cooked quickly (FAST) with a grill (Vulcan commercial gas grill model VCCV 36-1; Vulcan Hart Corp., Louisville, KY) temperature of 249°-260°C to an internal temperature of 63°C and brought to 65°C during a 1 h hold in a commercial foodservice warming oven (Precision RS-201, Metal Products, Inc, Miami, FL) kept at approximately 74°C. A second steak from the muscle was slow cooked (SLOW) with a grill temperature of 149°C to an internal temperature of 63°C and held for 1 h to a final internal temperature of 65°C. The remaining two steaks from each muscle were cooked SLOW and FAST, respectively, to an internal temperature of 65°C and served with no holding time (0 h). Steaks to be served with no holding time were timed to finish cooking near the end of the 1 h holding period of the other two steaks. To reduce charring, samples were cooked for two min, flipped, cooked for another two min, and then flipped every min until the desired internal temperature was met. Weight losses from cooking and holding were determined. The steaks with 0 h holding time were placed in a double broiler (< 5 min) after cooking to maintain temperature prior to serving.

In order to prevent bias, panelists were seated in individual booths equipped with red fluorescent lights and partitioned to reduce possible collaboration between panelists and eliminate visual differences. An exhaust fan was turned on to create negative air pressure so aromas from cooking would not influence the panelists’s response. Each panelist was served distilled water and unsalted, saltine crackers and given three minutes between samples to cleanse their palates. Samples were served on ceramic plates through a bread-basket partition. The panel evaluated the 1.25 cm x 1.25 cm x 2.54 cm pieces of
the eight steaks each session for tenderness, connective tissue, juiciness, and off-flavor intensity on an 8-point hedonic scale (Appendix 1).

**Chemical Analysis**

Muscle samples were cubed, frozen in liquid nitrogen, and pulverized with a Waring blender (Waring Products Division, New Hartford, CT). Pulverized samples were stored at -80°C and used for moisture and ash analysis with a LECO Thermogravimetric Analyzer-601 (Model 604-100-400, LECO Corp., St. Joseph, MI) with a TGA-601 Windows (version 1.2, LECO corp., St. Joseph, MI) option. Eighteen samples (9 samples in duplicate) were run simultaneously according to the following table.

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<td>130°C</td>
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<tr>
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<table>
<thead>
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<td>0.05%</td>
<td>0:09 min</td>
</tr>
<tr>
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<td>0.05%</td>
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**General Settings**

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<td>Ash</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moisture</td>
</tr>
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</table>

Data were captured in a spreadsheet-based program (LECO version 1.23 software, LECO Corp., St Joseph, MI) that calculates percentage of moisture and ash, and reported values are an average of the duplicates. Crucibles were washed in soapy and distilled water and dried in a drying oven (100°C) for at least 4 h. Dried crucibles were stored for future use in a desiccator to cool to room temperature.
To determine pH, 10 g of pulverized sample in a 250 mL plastic beaker was homogenized at 10,800 rpm (speed 5) for 30 sec with 90 mL of double distilled water using a Polytron blender (Brinkman Instruments, New York, NY). A stir bar was placed in the beaker and placed on a stir plate. The calibrated (4 and 7) pH probe (Orion model 9256 BN, Orion Research, Inc. Boston, MA) was placed in the stirring mixture to determine the pH with an Orion SA 720 pH meter (Orion Research, Inc. Boston, MA). The probe was rinsed between each sample with double, distilled water and wiped dry with a Kimwipe (Kimberly-Clark Corp., Roswell, GA).

Total heme-iron concentration was determined using the method of Hornsey (1956) as modified by Lee et al. (1998). Two grams (±0.01 g) of pulverized sample were weighed into 50 mL plastic centrifuge tubes in triplicate. Tubes were wrapped with aluminum foil to reduce light exposure. All samples were weighed the day before the samples were run, stored in -20°C freezer, and covered with parafilm and foil. Samples were pulled from the freezer and, enough water, based on sample moisture percentage, was added so each sample was 72% moisture. The samples were homogenized at 10,800 rpm (speed 5) using a Polytron (Brinkman Instruments, New York, NY) with 8.1 mL of acetone and 0.2 mL of hydrochloric acid. This mixture was filtered through #2 Whatman filter paper (90 mm in diameter). After eight samples were filtered, the tubes were stored for approximately 15 minutes in a dark cabinet to limit light exposure. The filtrate was then read using a Cary 100 Varian UV/Visual Spectrophotometer (Varian Instruments, Sugarland, TX) at an absorbance of 640 nm. The absorbance value was then multiplied by 680 to give the amount of total pigment. Total pigment was used to calculate heme-iron (total pigment x 8.82/100).
Statistical Analysis

Treatments were allocated in a factorial arrangement, and data were analyzed as a randomized complete block design by analysis of variance (ANOVA) using the GLIMMIX procedure of SAS (Version 9.1.3, SAS Institute, Inc., Cary, NC) with a predetermined significance level of $P \leq 0.05$. Muscle served as the experimental unit while animal was a blocking factor and was considered a random effect. The Kenward-Roger option was used to determine denominator degrees of freedom. Main effects of muscle, cooking rate, and holding time and their two-way and three-way interactions were included in the model. When two-way interactions were significant, simple effects were generated by the SLICE and SLICEDIFF function in SAS, but when three-way interactions were significant, simple effects were generated by the DIFF and LINES functions in SAS. When main effect significance was indicated by ANOVA, mean separations were performed using the least squares means and DIFF functions of SAS.

The TER, VAI, and VAM were not evaluated for cooking rate due to sample size. These three muscles were separated from the other muscles and analyzed as mentioned previously, without including cooking rate or their interactions in the model.

Percentages of panelists detecting specific off-flavors were calculated for each individual muscle and analyzed as previously described.

STUDY 2

Protocol for Determining Volatile Compound Differences Between Liver-Like and Normal Beef Samples Using Gas Chromatography

This study was conducted from April-May, 2005 and August-November, 2005.
Sample preparation

From the previous study, off-flavored and normal steaks were identified from *M. infraspinatus*, *M. teres major*, *M. triceps brachii* long head, *M. rectus femoris*, *M. vastus lateralis*, *M. vastus medialis*, and *M. vastus intermedius*. Samples rated as five or above on an 8-point hedonic scale were considered normal. Samples that were rated as off-flavored (below five on an 8-point scale) and identified as being liver-like by a majority of the panelists were used in the initial compound collection study.

Powdered samples from the previous study were initially used to find collection times as well as collecting some samples and running through the gas chromatograph (GC). However, samples ran low so most of the work was conducted and validated with the *M. rectus femoris* since there was a remaining steak and more powdered sample from the first study. A remaining uncooked steak from one normal and one liver-like *M. rectus femoris* from USDA Select carcasses was homogenized by slicing the steak into smaller pieces, freezing the steak in liquid nitrogen, and pulverizing with a Waring blender (Waring Products Division, New Hartford, CT). Samples were maintained in a -80°C freezer until analyzed. To try to conserve samples to have a final complete comparison between the muscles, the final protocol was developed with the *M. rectus femoris*, as the other samples ran low.

Collection of Volatile Compounds

Five grams of raw powdered meat samples from the *M. infraspinatus*, *M. teres major*, *M. triceps brachii* long head, *M. rectus femoris*, *M. vastus lateralis*, *M. vastus medialis*, and *M. vastus intermedius* and 100 mL of distilled water were mixed in a glass gas dispersion flask (Figure 1; p. 158) and flushed with nitrogen gas in a 40°C water
bath. Times for the release of off-odors were collected using nitrogen gas flow rates of 54.5 mL/min or 150 mL/min. The author would sniff the air leaving the flask and mark down the times undesirable odors were smelled. This was repeated at least twice for each sample for both normal and liver-like samples. Additional runs with the *M. rectus femoris* verified the times of undesirable smells coming off the solution. For collection, the 150 mL/min nitrogen gas flow rate was used with the *M. rectus femoris*. Volatile compounds were trapped in a packed porous polymer (20331 Porapak Q 80-100 mesh, matrix; SUPELCO, Bellefonte, PA) column, pre-washed with ethyl ether, attached to the outlet of the gas dispersion flask for 0-5 min. A second column was attached for 5-10 min. The packed column was a glass Pasteur pipette with glass wool stuffed in the bottom, 5 g of Porapak Q, and glass wool packed in the top. The columns were stored in a glass jar with desiccant until use. In the initial runs with the other muscles, the packed column was not pre-washed with ethyl ether so there was a lot of background noise in the chromatograms. The ends of all columns were quickly wrapped with parafilm after removal from the glass gas dispersion container and stored in a container with desiccant to ensure no additional components were absorbed.

The columns were flushed with ethyl ether (2 mL); extracted samples were captured and stored in a glass vial at -20°C for no more than 1 d.

**Gas chromatography**

Ether extracts were splitlessly injected (1 μL) in a GC (Hewlett-Packard 6890 Series- GC System, Agilent Technologies, Santa Clara, CA) with oven conditions starting at 35°C, raising 2°C/min until 200°C was reached, and held ten min for a 92.50 min total run time. A 30 m, 5% phenyl-95% methylpolysiloxane GC column (Alltech Capillary
Column ECTM-5, Alltech Associates, Inc, Deerfield, IL) with an inner diameter of 0.32 mm and film thickness of 0.25 μm was used. Gas flow rate was 24.7 mL/min, and the injection and FID temperature were 250°C. The final run with all the correct parameters was only run in duplicate on one liver-like sample and one normal sample.

After the initial protocol was developed, an attempt to capture the compounds coming off the GC was carried out to try to capture just the undesirable smells. The FID was turned off and an olfactory device was made and attached to the detector port. The olfactory device was an attachment that fit in the detector outlet with tubing extending from the outlet with a needle attached. As compounds came off the column, the author would take note of the smell being emitted through the needle and the time, with the idea that when peaks of off-flavored smells came off, they could be captured in liquid nitrogen and taken to a mass spectrometer for identification. This was repeated five times in one day with a 20-30 min break between runs.

**Mass spectrometry**

A portion of each ether-extracted sample in a glass vial was sent for identification of compounds by mass spectrometry to the University of Nebraska Water Resources Lab. The samples were injected with a 50:50 split into a GC-Mass Spectrometer (MS) system (Hewlett-Packard GC 6890, Agilent Technologies, Santa Clara, CA; Hewlett Packard MS 5973, Agilent Technologies, Santa Clara, CA). The column (Hewlett-Packard 5MS, Agilent Technologies, Santa Clara, CA) used was 30 m in length with 0.25 mm ID and 0.25 μm film thickness. Identification was made using the NIST/EPA/NIH Mass Spectral Library (Version 2.0a, build July 1, 2002).
STUDY 3

Identification of Volatile Compounds in Beef Round and Chuck Muscles

This project’s samples were analyzed on Dec 14-16, 2005; Feb 1-15, 2006; March 15-22, 2006; and May 15-19, 2006.

Justification for the Purge and Trap Method

The preceding GC protocol did not allow for identification of compounds with the MS due to the ethyl ether and the limited concentration of the compounds. The UNL Water Resources Lab bought a purge and trap system while continued efforts to refine the protocol were taking place. Therefore, efforts to modify the preceding methods were halted since the purge and trap system eliminates several steps where compounds may be lost or changed due to other chemicals or excessive handling.

Purge and Trap Mass Spectrometry

The M. infraspinatus (Flat Iron; INF), M. triceps brachii (Clod Heart; TRI), M. rectus femoris (Knuckle Center; REC), M. vastus lateralis (Knuckle Side; VAL), and M. vastus intermedius (Knuckle Bottom; VAI) were evaluated in the first study. These muscles from USDA Select carcasses were identified as ‘liver–like’ or ‘normal’ by a trained taste panel with ‘normal’ classification having an off–flavor rating of five or above on an 8–point scale. Comparable, normal INF were not available at the time of this testing, but the four other muscles had 2-3 liver-like samples and at least two normal samples tested. Five grams of raw, pulverized sample were weighed into 50 mL glass injection vials and maintained frozen (-80°C) until run (< 2 d). No more than four frozen samples were placed in an autosampler (O-I-Analytical Water/Soil Autosampler, Model 4552, College Station, TX) for analysis so samples would not be held for longer
than 2.5 h before analyzing. Ten mL of distilled water were added to each vial prior to that sample’s analysis. The purge and trap system (O-I-Analytical Eclipse, Model 4660, College Station, TX) flushed the sample with helium gas and heated the sample to 40 or 80°C to allow the volatiles to separate from the sample. (The purging process involved flushing the sample with purified helium and heating the samples to remove the volatiles). Purge time was 11 min at 30°C in a Trap10 trap (O-I-Analytical, College Station, TX). This trap is recommended for use with mass spectrometry. It has equal length of Tenax, silica gel, and carbonized molecular sieve to give equivalent trapping and desorb performance with lower background level of carbon dioxide (O-I-Analytical Application Note 12861198). After purging, the volatiles were desorbed 4 min at 190°C. The subsequent run on the GC/MS (Agilent Technologies 6890N Network GC system) used a 30 m. 0.25 mm ID, and 0.25 μm film thickness column. The volatile compounds were held at 40°C for 4 min followed by an 8°C/min ramp to 250°C and held for 10 min. Compound masses were then determined with a mass spectrometer (Agilent Technologies 5973 inert Mass Selective Detector) and identified by the database (NIST/EPA/NIH Mass Spectral Library, Version 2.0a).

A list of times and possible compounds were made for each sample. Comparisons between like samples were made, and a list was compiled of each compound. Compounds with a quality (how close the compound matches up with a compound in the database) of less than 50% were considered an unknown compound unless another like sample had the peak at the same retention time and estimated concentration with a quality of greater than 70%. Relative comparisons of volatile compounds between liver-like and normal samples were made by estimating the peak height from the liver-like samples and
the normal samples and then visually determining if there was a difference. Determination of the differences between normal samples from one muscle in the chuck and normal samples obtained from three muscles from the knuckle were done in the same manner.

**STUDY 4**

**Uncooked Beef Muscles with Liver-Like Flavor are Similar in Volatile Compounds to Raw Beef Liver**

This project’s samples were run on March 15-22, 2006.

One over-wrapped film package of raw liver was purchased from a local supermarket, minced, frozen in liquid nitrogen, and pulverized with a Waring blender (Waring Products Division, New Hartford, CT). Pulverized *M. infraspinatus*, *M. rectus femoris*, *M. triceps brachii*, and *M. vastus lateralis* that had been identified as liver-like from the first study were used for comparison. The results from the third study were the compounds used as comparison to the liver samples.

Five grams of raw, pulverized sample were weighed into 50 mL glass injection vials and maintained frozen (-80°C) until run (< 2 d). Samples were placed in an autosampler (O-I-Analytical Water/Soil Autosampler, Model 4552) to maintain proper temperature. Ten mL of distilled water was added to each vial prior to each sample’s analysis. The purge and trap system (O-I-Analytical Eclipse, Model 4660) heated the sample to 40 or 80°C to allow the volatiles to separate from the sample. Purge time was 11 min at 30°C in a Trap10 trap (O-I Analytical, College Station, TX). After purging, the volatiles were desorbed 4 min at 190°C. The subsequent run on the GC/MS (Agilent Technologies 6890N Network GC system) used a 30 m, 0.25 mm ID, and 0.25 μm film thickness column. The volatile compounds were held at 40°C for 4 min followed by an
8°C/min ramp to 250°C and held for 10 min. Compound masses were then determined with a mass spectrometer (Agilent Technologies 5973 inert Mass Selective Detector) and identified by the database (NIST/EPA/NIH Mass Spectral Library, Version 2.0a).

Relative comparisons of volatile compounds between raw beef liver and raw liver-like beef muscle samples were made as in the third study.

**STUDY 5**

**Validation of the Purge and Trap Mass Spectrometer Results with SPME using *M. triceps brachii* and Verification of Compounds with *M. rectus femoris**

The purge and trap samples were run May 15-19, 2006 and May 30-31, 2006 while the solid phase microextraction was run at North Dakota State University in the summer of 2006.

Due to the unavailability of pulverized muscles in which a sensory evaluation had been conducted on a corresponding steak, the same muscle was not used for the validation and verification.

**SPME validation**

Solid phase microextraction was run according to Fernando, Berg, & Grun (2003). Briefly, 0.5 g pulverized *M. triceps brachii* (n=2 liver-like; n=2 normal) that had been identified by a trained taste panel as normal or liver-like in flavor from the first study were weighed into 4 mL vials with 1.0 g of distilled water and vortexed for 10 sec. The samples were incubated at 90°C for 10 min and vortexed for 10 sec. The samples were allowed to absorb onto the filament (57328-U; 50/30 DVB/CAROXEN/PDMS Stable Flex; Supleco) in a 50°C water bath, and desorbed on the gas chromatogram (GC)
inlet for 5 min. Injector temperature was 250°C while the detector temperature was 275°C. The oven parameters were set to run at 35°C for 5 min, increase 8°C/min to a temperature of 75°C, followed by a ramp of 40°C/min to 200°C and held for 5 min.

Hexanal, pentane, and propanal were run as standards while other compounds from the SPME method were identified by mass spectrometry. Relative quantitative comparisons for normal and liver-like samples were only done on hexanal, propanal, and pentane because the SPME protocol used only indicated the presence or absence of compounds. Estimates of concentration were not taken as in study 3 so some of the compounds that were present in both samples may have been in different concentration even though not indicated in the results. Results were compared to compounds identified in the third study.

**Verification of compounds found in the M. rectus femoris with Purge and Trap MS**

Six (3 USDA Choice; 3 USDA Select) *M. rectus femoris* from the taste panel portion of the innovative selection procedure of Jenschke et al. (2006) were selected to verify the presence of volatile compounds that were used to compile the list of volatile compounds from raw muscle samples in study 3. None of the samples chosen were found to be liver-like (1 or higher on a 15-point scale) by taste panelists (Jenschke et al., 2006). Five grams of raw, pulverized sample were weighed into 50 mL glass injection vials and maintained frozen (-80°C) until run (< 2 d). Samples were placed in an autosampler (O-I-Analytical Water/Soil Autosampler, Model 4552, College Station, TX) to maintain proper temperature as well as add 10 mL of distilled water to each vial prior to its run. The purge and trap system (O-I-Analytical Eclipse, Model 4660, College Station, TX) heated the sample to 80°C to allow the volatiles to separate from the sample.
Purge time was 11 min at 30°C in a Trap10 trap (O-I Analytical, College Station, TX). After purging, the volatiles were desorbed 4 min at 190°C. The subsequent run on the GC/MS (Agilent Technologies 6890N Network GC system) used a 30 m x 0.25 mm ID x 0.25 μm film thickness column. The volatile compounds were held at 40°C for 4 min followed by an 8°C/min ramp to 250°C and held for 10 min. Compound masses were then determined with a mass spectrometer (Agilent Technologies 5973 inert Mass Selective Detector) and identified by the database (NIST/EPA/NIH Mass Spectral Library, Version 2.0a).
References


The Influence of Cooking Rate and Holding Time on Beef Chuck and Round Flavor

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ABSTRACT

Steaks from seven muscles from ten beef carcasses were cooked quickly or slowly and held 0 or 1 h to explore the influence of cooking rate and holding time on beef flavor. Moisture, ash, pH, and heme iron concentration were determined for each muscle. Trained sensory panels evaluated the steaks for tenderness, juiciness, connective tissue, and off-flavor intensity in addition to identifying specific off-flavors. Off-flavor intensity was lowest when beef was cooked slowly (on a 149°C gas grill instead of a 249°C grill) and when it was held for 1 h prior to sensory evaluation. The M. infraspinatus had the least intense off-flavor and the M. vastus intermedius had the most intense off-flavor. Slow cooking or holding for 1 h prior to consumption reduced the intensity of off-flavor in value cuts from the beef chuck and round while chemical characteristics did not contribute to off-flavor in this study.

Keywords: Beef, Cooking Rate, Holding Time, Off-Flavors
1. Introduction

The beef chuck and round represent around fifty-three percent of the carcass. Due to recent research, supply, and marketing campaigns, the value of the beef carcass has increased approximately $15/cwt since 1998. As much as $6/cwt is attributed to the fabrication of steaks from individual muscles from the chuck (National Cattlemen, 2004) that were shown to have acceptable palatability attributes in the muscle profiling study (Von Seggern, Calkins, Johnson, Brickler, & Gwartney, 2005). Numerous studies have shown several muscles from the chuck and round to be tender (McKeith, De Vol, Miles, Bechtel, & Carr, 1985; Johnson, Chen, Muller, Costello, Romans, & Jones, 1988; Elam, Brooks, Morgan, & Ray, 2002b; Belew, Brooks, McKenna, & Savell, 2003; Kukowski, Maddock, & Wulf, 2004) and acceptable in overall palatability (McKeith, et al., 1985; Elam, Brooks, Morgan, & Ray, 2002a; Elam, Brooks, Morgan, & Ray, 2002b; Kukowski et al., 2004) by consumers and trained panelists. Therefore, the industry term value cuts refers to the beef muscles, mainly from the chuck and the round, that are being considered for utilization other than ground beef or roasts.

The foodservice industry has begun to use various steaks obtained from the chuck and the round. Managers in this industry report an increasing number of complaints about off-flavors in some of the value cuts from the beef chuck and round. Some of the typical off-flavors are described as liver-like, fatty, sour, and metallic. Research has been conducted that investigated the effect of quality grade (Yancey, 2002), aging (Calkins, 2002b; Yancey, 2002), marination (Streff, Wulf, & Maddock, 2003), degree-of-doneness (Adhikari, Keene, Heymann, & Lorenzen, 2004; Calkins, 2002a; Streff et al. 2003), and
cooking methods (Adhikari et al. 2004; Miller, 2001) on the occurrence of off-flavors. Other research has investigated the different biochemical characteristics that might cause the undesirable flavor. Yancey (2002) looked at lipid oxidation factors, such as fatty acids and sarcoplasmic proteins, which might lead to liver-like flavors and found that myoglobin concentration seemed to be related to the liver-like flavor.

Flavor is a combination of aroma and taste. As a result, some of the compounds that are part of the normal beef flavor may be concentrated or lost due to cooking. Goodson et al. (2002) discovered that flavor was the driving factor for overall acceptance ratings of clod steak (IMPS #114; NAMP 1997) in an in-home beef study. In the foodservice industry, meat is cooked and then traditionally held for a period of time before being served. Cuts commonly used in foodservice (e.g., prime rib, ribeye, and top butt steaks) are able to withstand this preparation method. With the less expensive beef value cuts being offered as substitutes for some of the main meat entrees, the impact of holding after cooking on flavor and other palatability issues needs to be addressed to ensure that customers are having pleasant eating experiences and are willing to purchase the product again. A previous study on beef roasts investigated the role cooking rate, holding time, fat trim level, endpoint temperature and oven type on flavor attributes and microbial levels (Belk, Miller, Evans, Liu, & Acuff, 1993). Endpoint temperature and oven type played the biggest role in flavor development, while cooking rate, fat trim level, endpoint temperature, and oven type influenced the perception of liver-like aromatics.
Therefore, the objective of this study was to determine the role of cooking rate and holding time on sensory characteristics of beef muscles from the chuck and round.

2. Materials and methods

2.1 Muscle preparation

Seven muscles (M. infraspinatus - INF, flat iron; M. teres major - TER, shoulder tender; M. triceps brachii - TRI, clod heart; M. rectus femoris - REC, knuckle center; M. vastus lateralis - VAL, knuckle side; M. vastus medialis - VAM, knuckle bottom; and the M. vastus intermedius - VAI, knuckle soft) located in the clod (IMPS #114; NAMP 1997) and knuckle (IMPS #167; NAMP 1997) from ten animals (5=Choice and 5=Select) were separated and trimmed of external fat after aging 7 d post-harvest. A 100 g portion of the proximal end of each muscle was removed and minced for chemical analysis. The thick band of connective tissue running through the center of the INF was removed. The TRI, REC, and VAL were cut into 2.54 cm steaks. The top and bottom portions of the INF were cut in half to make four steaks. The TER, VAM, and VAI were cut in half. Steaks were wrapped in freezer paper and frozen (-16°C) until sensory evaluation was conducted.

2.2 Sensory analysis

Panelists for this study were selected and trained according to the guidelines and procedures outlined by the AMSA (1995). They were recruited from an advertisement in a local paper as well as among the staff in the department. Recruits were screened for the tastes of sour, sweet, bitter, and salty. During training, panelists were presented with
samples with varying degrees of tenderness, juiciness, and connective tissue in order to anchor them to the scale. Panelists were trained to identify the presence of specific off-flavors (liver-like, metallic, sour, charred, oxidized, rancid, or other) contributing to the off-flavor score for the steak.

Four steaks from one USDA Choice and four steaks from one USDA Select muscle type were randomly served during every taste panel session. Serving order of muscles was randomized. Steaks were thawed 24 h prior to cooking for sensory evaluation. One steak from each muscle was cooked quickly (FAST) with a grill (Vulcan commercial gas grill model VCCV 36-1; Vulcan Hart Corp., Louisville, KY) temperature of 249°-260°C to an internal temperature of 63°C and brought to 65°C during a 1 h hold in a commercial foodservice warming oven (Precision RS-201, Metal Products, Inc, Miami, FL) kept at approximately 74°C. A second steak from the muscle was slow cooked (SLOW) with a grill temperature of 149°C to an internal temperature of 63°C and held for 1 h to a final internal temperature of 65°C. The remaining two steaks from each muscle were cooked SLOW and FAST, respectively, to an internal temperature of 65°C and served with no holding time (0 h). Steaks to be served with no holding time were timed to finish cooking near the end of the 1 h holding period of the other two steaks. Cooking times and weight losses from cooking and holding were determined.

In order to prevent bias, panelists were seated in individual booths equipped with red fluorescent lights and partitioned to reduce possible collaboration between panelists and eliminate visual differences. Each panelist was served distilled water and unsalted, saltine crackers and given three minutes between samples to cleanse their palates. The
panel evaluated the 1.25 cm x 1.25 cm x 2.54 cm pieces of the eight steaks each session for tenderness, connective tissue, juiciness, and off-flavor intensity on an 8-point hedonic scale. The panel also identified specific off-flavors.

2.3 Chemical analysis

Muscle samples were cubed, frozen in liquid nitrogen, and pulverized with a Waring blender (Waring Products Division, New Hartford, CT). Pulverized samples were stored at -80°C and used for moisture and ash analysis with a LECO Thermogravimetric Analyzer-601 (Model 604-100-400, LECO Corp., St. Joseph, MI) with a TGA-601 Windows (version 1.2, LECO corp., St. Joseph, MI) option.

To determine pH, 10 g of pulverized sample was homogenized at 10,800 rpm for 30 sec with 90 mL of double distilled water using a Polytron blender (Brinkman Instruments, New York, NY). A calibrated (4 and 7) pH probe (Orion model 9256 BN, Orion Research, Inc. Boston, MA) was placed in the stirring mixture to determine the pH with an Orion SA 720 pH meter (Orion Research, Inc. Boston, MA).

Total heme-iron concentration was determined using the method of Hornsey (1956) as modified by Lee, Hendricks, & Cornforth (1998). Two grams (±0.01 g) of pulverized sample were weighed into tubes and concentration was determined in triplicate. Samples were homogenized using a Polytron (Brinkman Instruments, New York, NY) with 8.1 mL of acetone and 0.2 mL of hydrochloric acid. This mixture was filtered through #2 Whatman filter paper (90 mm in diameter). After eight samples were filtered, the tubes were stored for approximately 15 minutes in a dark cabinet to limit light exposure. The filtrate was then read using a Cary 100 Varian UV/Visual
Spectrophotometer (Varian Instruments, Sugarland, TX) at an absorbance of 640 nm. The absorbance value was then multiplied by 680 to give the amount of total pigment. Total pigment was used to calculate heme-iron (total pigment x 8.82/100).

2.4 Statistical analysis

Treatments were allocated in a factorial arrangement, and data were analyzed as a randomized complete block design by analysis of variance (ANOVA) using the GLIMMIX procedure of SAS (Version 9.1.3, SAS Institute, Inc., Cary, NC) with a predetermined significance level of $P \leq 0.05$. Muscle served as the experimental unit while animal was a blocking factor and considered a random effect. The Kenward-Roger option was used to determine denominator degrees of freedom. Main effects of muscle, cooking rate, and holding time and their two-way and three-way interactions were included in the model. When two-way interactions were significant, simple effects were generated by the SLICE and SLICEDIFF function in SAS, but when three-way interactions were significant, simple effects were generated by the DIFF and LINES functions in SAS. When main effect significance was indicated by ANOVA, mean separations were performed using the least squares means and DIFF functions of SAS.

The TER, VAI, and VAM were not evaluated for cooking rate due to sample size. These three muscles were separated from the other muscles and analyzed as mentioned previously, without including cooking rate or their interactions in the model.

Percentages of panelists detecting specific off-flavors were calculated for each individual muscle and analyzed as previously described.
3. Results and discussion

3.1 Chemical Analysis

The TRI and VAL had the lowest pH, and the VAI had the highest (Table 1). The muscles from the chuck and the REC had lower pigment and heme iron concentration ($P < 0.05$). The VAM was found to have the highest moisture percentage ($P < 0.05$) while the VAI and INF had the lowest ash values ($P < 0.05$). The same trends were seen in the muscling profiling study (Von Seggern et al., 2005) although no relationships were established in this study between the tested chemical characteristics and off-flavor perception.

3.2 Cooking, Holding, and Total Loss

Table 2 shows the cooking and holding loss for the TER, VAI, and VAM, muscles that were all FAST cooked with two holding times. The samples held for 1 h had less cook loss ($P < 0.0001$) than those held for 0 h. This was expected as the steaks cooked with 0 h hold were cooked to the final temperature while the 1 h hold steaks were allowed to reach the final temperature in the holding oven. The steaks held for 1 h had hold loss, with the TER having the least hold loss (10.14%; $P < 0.05$) compared with the VAI (14.33%) and VAM (15.40%). Total loss revealed that the 1 h hold had a higher percentage total loss ($P < 0.0001$) than steaks that were not held. The specific muscle did not affect cooking loss or total loss for the TER, VAI, and VAM.

The INF, TRI, REC, and VAL steaks were cooked FAST or SLOW and held 0 or 1 h. Cooking and holding losses are shown in Table 3. As seen in the other three muscles, the 0 h holding time had more cook loss than the 1 h holding time steaks since
the 0 h holding time steaks were cooked to the final desired temperature. The SLOW steaks had a higher percentage of cook loss as well. This was attributed to the increased time on the grill compared to the FAST steaks (Table 4). The TRI had the lowest cooking loss percentage while the REC had the most. There was a holding time x cooking rate interaction for holding time loss. The FAST steaks with a 1 h holding time had a higher percentage of holding loss than the SLOW steaks with 1 h holding time. The SLOW steaks lost more weight during the cooking than the FAST steaks so the difference seen in the holding time loss is the available water that was still in the FAST steaks. The holding time x muscle interaction for holding time follows the same trend seen with the holding time x cooking rate interaction. The steaks held 1 h had a higher percentage of loss compared to the steaks held 0 h. The TRI steaks held 1 h had the highest percentage of hold loss, but had the lowest cook loss whereas the REC had the highest cook loss and minimal hold loss. For total percentage loss, the FAST and SLOW steaks that were held 1 h had the most loss and were not significantly different ($P = 0.3128$). The SLOW steaks with no holding time had more loss than the FAST steaks with no holding time due to the increased time on the grill. Each of the four muscles had a higher percentage of total loss when the steaks were held 1 h. The REC steaks held 1 h had the lowest percentage total loss of the four muscles that had steaks held for 1 h even though it had an average amount of moisture determined by proximate analysis.

3.3 Sensory Analysis

The TER, VAI, and VAM were too small to obtain four steaks from the muscle so only the FAST cooking rate was used for these muscles. There were no muscle x holding
time interactions for tenderness, connective tissue, or off-flavor intensity (Table 5).

Compared to the VAI and VAM, the TER was rated as much more tender ($P < 0.0001$) with less connective tissue ($P < 0.0001$). The VAI was perceived to have the most intense off-flavor of the three muscles. Numerically, all steaks from muscles served with no holding time received higher juiciness scores than when the steaks had 1 h holding time. However, only the VAM steaks with 1 h holding were significantly lower ($P < 0.0001$) in juiciness ratings.

Other studies have evaluated the palatability of steaks from the beef chuck and round. These studies have found that the *M. teres major* received high ratings for tenderness (Meisinger, James, & Calkins, 2006; Elam et al., 2002a) and overall acceptability (Elam et al. 2002a) as well as low amounts of connective tissue (Meisinger, et al. 2006).

Calkins (2002a) found the *M. teres major* received the lowest overall flavor preference, at slightly undesirable, for the muscles tested and was in the lower third of the muscles tested in Meisinger et al. (2006) study for off-flavor intensity. The specific off-flavor that appeared to contribute to the TER off-flavor intensity rating was sourness (Meisinger, et al., 2006). This study also demonstrated that while the TER rated well in other attributes, the off-flavor intensity fell just below five on the 8-point scale. With an 8-point hedonic scale, typically a rating below five is said to be unacceptable. The VAM rated slightly above five, but not significantly different from the TER.

The INF, TRI, REC, and VAL were analyzed with both cooking rate and holding time. The INF was found to be the most tender (Table 6), TRI and REC were similar in trained tenderness ratings, and VAL was considered to fall between slightly tough and
slightly tender on the 8-point scale. There were no differences in tenderness due to holding time \( (P = 0.5269) \) or cooking rate \( (P = 0.9618) \). Numerous studies have shown that the INF is one of the most tender muscles in the beef carcass as evaluated by both Warner Bratzler Shear Force (WBSF) (Belew, et al., 2003; Von Seggern et al., 2006) and through sensory evaluations (Calkins, 2002a; Calkins, 2002b; Elam et al. 2002a; Elam et al. 2002b; Meisinger et al., 2006;), which was demonstrated again in this study. The other muscles also fell in line with previous ratings and WBSF values.

The muscle x holding time interaction led to a perceived difference in the amount of connective tissue detected by the panelists. However, no trend can be seen from the interaction. One would expect a longer holding time at the cooked temperature would allow further breakdown of collagen, but this was not seen. The VAL, with either holding time, had the most connective tissue of the four muscles. The INF held for 1 h was rated as having the least amount of connective tissue. The effect of cooking rate on connective tissue ratings was approaching significance \( (P = 0.0765) \) as those cooked FAST were rated numerically as having less connective tissue.

Steaks from the four muscles with 0 h holding time always had higher numerical juiciness ratings than those steaks with 1 h holding time (Table 7). Statistically, only the TRI and VAL showed significant \( (P < 0.0001) \) differences between the holding times. Contrary to expectations, the SLOW REC and VAL were perceived to be more juicy \( (P = 0.0500 \text{ and } 0.0528, \text{ respectively}) \) than the steaks cooked FAST. The TRI steaks were approaching significance \( (P = 0.0748) \) with the FAST being rated as more juicy than the SLOW. The INF had no difference \( (P = 0.9491) \) due to cooking rate for juiciness.
Interestingly, the steaks cooked FAST and held for 1 h were perceived to be less juicy than the steaks cooked SLOW and held for 1 h even though total cook loss was the same for both samples. FAST and SLOW steaks held 0 h were similar ($P = 0.3434$) for juiciness ratings although FAST steaks (24.54 %) had less actual total cooking loss than SLOW steaks (27.83 %). Carmack, Kastner, Dikeman, Schwenke, & Garcia Zepeda (1995) found a much larger difference in juiciness in the INF compared to the TRI and REC than was seen in McKeith et al. (1985) and this study although the final endpoint temperature was lower in this study.

Off-flavor intensity scores for the INF, TRI, REC, and VAL had a three way interaction of muscle x cooking rate x holding time ($P=0.0101$). The FAST cook rate and held for 0 h had the poorest scores for off-flavor intensity for the TRI and VAL muscles (Table 8). The INF and the REC were not significantly different ($P > 0.05$) among the cooking rate and holding time treatments. In each muscle, except for the INF, the slow cook had a numerically higher score (indicating less off-flavor) than when the muscle was cooked FAST and held for the same amount of time. These results agree with Belk et al. (1993) when they evaluated beef roasts where they found all ratings for off-flavor aromatics were less intense when cooked at a slow rate. In contrast, Cremer (1986) found increases in aroma and flavor when turkey rolls were held for at least 120 min of time. While beef-flavor intensity was not evaluated in this study, Carmack et al. (1995) reported values of 7.3, 7.1, and 6.8 (on a 10 point scale) for the TRI, REC, and INF, respectively, for beef-flavor intensity although this did not correspond with flavor
desirability. McKeith et al. (1985) found that the INF was higher in flavor desirability than the TRI while the REC was not different from either muscle.

Table 9 illustrates that the TER, VAI, and VAM muscles had the same incidence of liver-like, metallic, charred, and other flavors as determined by the percentage of panelists denoting that specific off-flavor, regardless of hold time. The liver-like flavor may have been promoted since the samples were only cooked to a final endpoint temperature of medium rare which has been shown to increase perception of liver-like flavor in beef (Adhikari et al., 2004; Miller, 2002). The VAI had a lower percentage of panelists denoting a sour flavor, but a higher percentage for rancid and fatty flavors than the other two muscles. Holding time did not influence the percentage of panelists indicating specific flavors. Oxidized flavor was affected by the muscle x holding time interaction with the VAI held 1 h having the lowest (7.7%) percentage of panelists while VAM with 1 h holding time having the highest (23.2%). A slight warmed over effect might explain this condition.

Panelists found sourness at a higher frequency in the TRI and the VAL when comparing the four muscles that had the cooking rate and holding time treatments (Table 10). The INF and REC also had lower percentages of panelists indicating the samples were oxidized compared to the TRI and VAL. Due to the ‘plumping’ (increase in volume) of these muscles during grilling and the use of a commercial grill, some of the steaks were perceived to have a charred taste since the cooking time increased and the samples were in contact with the grill for longer periods of time. Holding time did not affect liver-like, metallic, sour, oxidized, fatty, or other flavors. Sour flavor was
perceived by a higher percentage of panelists when steaks were cooked SLOW (31.4% versus 25.6%). Metallic and fatty flavors had a muscle x cooking rate interaction. The INF cooked FAST had the lowest percentage (5.2%) of panelists indicating the steaks were metallic while the TRI cooked FAST had the highest percentage (16.6%). The INF, TRI, and REC cooked SLOW and the REC cooked FAST were similar to INF cooked FAST. The VAL SLOW and FAST cooked steaks were similar to the TRI cooked FAST for metallic as well. The INF cooked SLOW was the only treatment that was different for fatty. The fatty flavor was probably perceived more often due to increased cooking loss in the SLOW cooked steaks which concentrated the fat flavor components (SLOW 7.05% versus FAST 2.38%). The same trend was seen with the VAI having more samples perceived as fatty. According to Von Seggern (2000), both the INF (9.18% lipid) and VAI (8.43% lipid) had a higher lipid composition than the TER (5.25%), TRI (5.65%), REC (5.11%), VAL (4.44%), VAM (4.35%). The samples cooked with lower heat (SLOW) had much lower incidences of panelists identifying the samples as charred. This is probably due to the high heat the FAST samples were exposed to where the outsides became more well done before the center of the steak reached the appropriate temperature. Furthermore, the 0 h holding time FAST cooked steaks were on the grill for a longer period of time to reach the desired temperature, while the 1 h held steaks were allowed to gain the last five degrees in the warming oven so they were not exposed to the grill for as long a time period. The INF was found to have the highest response of no off-flavors in the samples tested (data not shown).
Contrary to these results, in beef roasts, Belk et al. (1993) found that liver-like aromatics were increased when samples were cooked fast while sourness and metallic ratings were not affected by cooking rate. Additionally, Adhikari et al. (2004) found in the chuck muscles they tested, the less juicy samples also had less livery samples as was seen here.

**IMPLICATIONS**

Cooking rate and holding time play a role in the intensity of off-flavor perceived in muscles from the chuck and round, especially when the steaks are cooked quickly and served immediately. The slower cooking or the longer hold time create more total loss in weight and reduce intensity of off-flavor.

**Acknowledgements**

1 This paper is a contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE 68583.

2 This project was funded in part by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen’s Beef Board and state beef councils by the National Cattlemen’s Beef Association.
References


Von Seggern, D. D. 2000. Physical and chemical properties of 39 muscles form the beef chuck and round. *MS Thesis*, University of Nebraska, Lincoln, NE.


Yancey II, E. J. (2002) Determination of factors causing livery flavor in steaks from the beef loin and chuck. *PhD Dissertation*. Kansas State University, Manhattan, KS.
Table 1. Chemical analysis of seven muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>pH</th>
<th>Pigment (ppm)</th>
<th>Heme Iron (ppm)</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
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<td>50.40a</td>
<td>72.01a</td>
<td>0.992a,b</td>
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<td>75.07c,d</td>
<td>1.090c,d</td>
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<td>53.69a,b</td>
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<td>VAM</td>
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<td>57.98b,c</td>
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SE 0.058 24.70 2.14 0.4121 0.023
P-value <0.0001 0.0009 0.0003 <0.0001 <0.0001

1 INF = M. infraspinatus; TER = M. teres major; TRI = M. triceps brachii; REC = M. rectus femoris; VAI = M. vastus intermedius; VAL = M. vastus lateralis; VAM = M. vastus medialis

a-d LS means within a column that do not have a common superscript letter differ (P < 0.05).
Table 2. Cooking and holding loss of *M. teres major*, *M. vastus intermedius*, and *M. vastus medialis*

<table>
<thead>
<tr>
<th>Effects</th>
<th>Cook Loss$^1$</th>
<th>SE</th>
<th>Hold Time Loss$^1$</th>
<th>SE</th>
<th>Total Loss$^1$</th>
<th>SE</th>
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<td>0.98</td>
<td>29.31</td>
<td>1.18</td>
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<td>$P$-value</td>
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<table>
<thead>
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<th>Hold Time (h)</th>
<th>Cook Loss$^1$</th>
<th>SE</th>
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<th>SE</th>
<th>Total Loss$^1$</th>
<th>SE</th>
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<td>0</td>
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<th>Muscle x Hold Time (h)</th>
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<th>Hold Time Loss$^1$</th>
<th>SE</th>
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<td>0.00$^c$</td>
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<td>VAI 1</td>
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<td>$P$-value</td>
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$^1$ Cook loss is the weight loss during cooking; Hold loss is the weight loss during the holding period. The 0 h holding time samples had no holding loss; Total loss is the total weight lost during the cooking and holding preparations.

$^2$ TER = *M. teres major*; VAI = *M. vastus intermedius*; VAM = *M. vastus medialis*

$^a,b$ LS means that do not have a common superscript letter differ ($P < 0.0001$).

$^c-e$ LS means that do not have a common superscript letter differ ($P < 0.05$).
Table 3. Cooking and holding loss of from *M. infraspinatus, M. triceps brachii, M. rectus femoris, and M. vastus lateralis*

<table>
<thead>
<tr>
<th>Effects</th>
<th>Cook Loss$^1$</th>
<th>SE</th>
<th>Hold Loss$^1$</th>
<th>SE</th>
<th>Total Loss$^1$</th>
<th>SE</th>
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<tr>
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<tr>
<td>FAST</td>
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<tr>
<td>SLOW</td>
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<tr>
<td>P-value</td>
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<td>Muscle x Holding Time (h)</td>
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<tr>
<td>INF 0</td>
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<tr>
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<td>TRI 0</td>
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<td>34.23$^e$</td>
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</tr>
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<td>0.00$^f$</td>
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<td>Slow 1</td>
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</table>

$^1$ Cook loss is the weight loss during cooking; Hold loss is the weight loss during the holding period. The 0 h holding time samples had no holding loss; Total loss is the total weight lost during the cooking and holding preparations.

$^2$ INF = *M. infraspinatus*; TRI = *M. triceps brachii*; REC = *M. rectus femoris*; VAL = *M. vastus lateralis*

$^3$ Fast = grill temperature of 249-260°C; Slow = grill temperature of 149°C.

$^a-e$ LS means for each effect within a column that do not have a common superscript letter differ ($P < 0.05$). $^f-h$ LS means for each effect within a column that do not have a common superscript letter differ ($P < 0.01$).
Table 4. Cooking times for muscles cooked FAST\textsuperscript{a} and SLOW\textsuperscript{a}

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cook Time (min)</th>
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</tr>
<tr>
<td>FAST</td>
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<td>SLOW</td>
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<td>\textit{P-value}</td>
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<tr>
<td><strong>Muscle\textsuperscript{bc}</strong></td>
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<td>INF</td>
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</tr>
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<td>TRI</td>
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<td>REC</td>
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<td>VAL</td>
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<td>1.10</td>
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</table>

\textsuperscript{a} FAST = grill temperature of 249-260°C; SLOW = grill temperature of 149°C.

\textsuperscript{b} INF = \textit{M. infraspinatus}; TRI = \textit{M. triceps brachii}; REC = \textit{M. rectus femoris}; VAL = \textit{M. vastus lateralis}; TER = \textit{M. teres major}; VAI = \textit{M. vastus intermedius}; VAM = \textit{M. vastus medialis}

\textsuperscript{c} Analyzed with four muscles (INF, TRI, REC, and VAL) that had both cooking rates

\textsuperscript{d} Analyzed with three muscles (TER, VAI, VAM) that only had FAST cooking rate
Table 5. Sensory evaluation\(^1\) of *M. teres major*, *M. vastus intermedius*, and *M. vastus medialis*

<table>
<thead>
<tr>
<th>Effects</th>
<th>Tenderness</th>
<th>Connective Tissue</th>
<th>Off-Flavor</th>
<th>Juiciness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle(^2)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TER</td>
<td>6.52(^b)</td>
<td>5.74(^d)</td>
<td>4.99(^f)</td>
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<td>VAI</td>
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<td>4.18(^c)</td>
<td>4.03(^e)</td>
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</tr>
<tr>
<td>VAM</td>
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<td>4.38(^c)</td>
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<td>P-value</td>
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<td>&lt;0.0001</td>
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<table>
<thead>
<tr>
<th>Hold Time (h)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
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<td>4.90</td>
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<td>SE</td>
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<td>0.34</td>
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<tr>
<td>P-value</td>
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<td>0.2208</td>
<td>0.1160</td>
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</table>

<table>
<thead>
<tr>
<th>Muscle x Hold Time (h)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>TER 0</td>
<td>6.02(^h)</td>
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</tr>
<tr>
<td>TER 1</td>
<td>5.85(^h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAI 0</td>
<td>6.08(^h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAI 1</td>
<td>5.94(^h)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VAM 0</td>
<td>5.89(^h)</td>
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<td></td>
</tr>
<tr>
<td>VAM 1</td>
<td>4.19(^g)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SE</td>
<td></td>
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<tr>
<td>P-value</td>
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<td></td>
<td>0.22</td>
<td>0.0019</td>
</tr>
</tbody>
</table>

\(^1\)Sensory ratings: 8 = extremely tender/no connective tissue/no off-flavor/extremely juicy, 7 = very tender/trace amount/trace off-flavor/very juicy, 6 = moderately tender/slight amount/slight off-flavor/moderately juicy, 5 = slightly tender/small amount/small off-flavor/slightly juicy, 4 = slightly tough/modest amount/modest off-flavor/slightly dry, 3 = moderately tough/moderate amount/moderate off-flavor/moderately dry, 2 = very tough/slightly abundant amount/very off-flavor/very dry, 1 = extremely tough/abundant amount/extreme off-flavor/extremely dry

\(^2\)TER = *M. teres major*; VAI = *M. vastus intermedius*; VAM = *M. vastus medialis

\(^a-h\) LS means within a column without a common superscript differ (*P* < 0.05)
Table 6. Sensory tenderness and connective tissue scores\(^1\) from *M. infraspinatus*, *M. triceps brachii*, *M. rectus femoris*, and *M. vastus lateralis*

<table>
<thead>
<tr>
<th>Effects</th>
<th>Tenderness</th>
<th>Connective Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle(^3)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INF</td>
<td>6.78(^c)</td>
<td></td>
</tr>
<tr>
<td>TRI</td>
<td>6.05(^b)</td>
<td></td>
</tr>
<tr>
<td>REC</td>
<td>5.83(^b)</td>
<td></td>
</tr>
<tr>
<td>VAL</td>
<td>4.83(^a)</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

| **Hold Time (h)**      |            |                   |
| 0                      | 5.90       |                   |
| 1                      | 5.84       |                   |
| SE                     | 0.10       |                   |
| **P-value**            | 0.5269     |                   |

| **Cooking Rate\(^2\)**|            |                   |
| Fast                   | 5.87       | 5.32              |
| Slow                   | 5.88       | 5.15              |
| SE                     | 0.10       | 0.12              |
| **P-value**            | 0.9618     | 0.0765            |

| **Muscle x Hold Time (h)**|            |                   |
| INF 0                    | 5.88\(^d\) |                   |
| INF 1                    | 6.32\(^e\) |                   |
| TRI 0                    | 4.94\(^b\) |                   |
| TRI 1                    | 5.16\(^b,c\)|                 |
| REC 0                    | 5.73\(^d\) |                   |
| REC 1                    | 5.30\(^c\) |                   |
| VAL 0                    | 4.17\(^a\) |                   |
| VAL 1                    | 4.40\(^a\) |                   |
| SE                       | 0.17       |                   |
| **P-value**              | 0.0119     |                   |

\(^1\) Tenderness/connective tissue scores: 8 = Extremely tender/no connective tissue, 7 = very tender/trace amount, 6 = moderately tender/slight amount, 5 = slightly tender/small amount, 4 = slightly tough/modest amount, 3 = moderately tough/moderate amount, 2 = very tough/slightly abundant, 1 = extremely tough/abundant

\(^2\) Fast = grill temperature of 249-260°C; Slow = grill temperature of 149°C.

\(^3\) INF = *M. infraspinatus*; TRI = *M. triceps brachii*; REC = *M. rectus femoris*; VAL = *M. vastus lateralis*

\(^a\)-\(^e\) LS means within a column without a common superscript differ (*P* < 0.05)
Table 7. Sensory juiciness scores\(^1\) from *M. infraspinatus*, *M. triceps brachii*, *M. rectus femoris*, and *M. vastus lateralis*

<table>
<thead>
<tr>
<th>Effects</th>
<th>Juiciness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle(^3) x Holding Time (h)</td>
<td></td>
</tr>
<tr>
<td>INF 0</td>
<td>5.73(^d)</td>
</tr>
<tr>
<td>INF 1</td>
<td>5.63(^d)</td>
</tr>
<tr>
<td>TRI 0</td>
<td>5.66(^d)</td>
</tr>
<tr>
<td>TRI 1</td>
<td>4.44(^b)</td>
</tr>
<tr>
<td>REC 0</td>
<td>5.71(^d)</td>
</tr>
<tr>
<td>REC 1</td>
<td>5.54(^d)</td>
</tr>
<tr>
<td>VAL 0</td>
<td>5.13(^c)</td>
</tr>
<tr>
<td>VAL 1</td>
<td>3.96(^a)</td>
</tr>
<tr>
<td>SE</td>
<td>0.16</td>
</tr>
<tr>
<td>(P)-value</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

| Muscle x Cooking Rate\(^2\) | |
| INF Fast | 5.68\(^{i,j}\) |
| INF Slow | 5.67\(^{i,j}\) |
| TRI Fast | 5.23\(^{g,h}\) |
| TRI Slow | 4.87\(^{f,g}\) |
| REC Fast | 5.43\(^{h,i}\) |
| REC Slow | 5.82\(^j\) |
| VAL Fast | 4.35\(^e\) |
| VAL Slow | 4.74\(^f\) |
| SE | 0.16 |
| \(P\)-value | 0.0229 |

| Holding Time (h) x Cooking Rate\(^2\) | |
| 0 Fast | 5.63\(^m\) |
| 0 Slow | 5.49\(^m\) |
| 1 Fast | 4.72\(^k\) |
| 1 Slow | 5.06\(^l\) |
| SE | 0.11 |
| \(P\)-value | 0.0189 |

\(^1\) Juiciness scores: 8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry, 1 = extremely dry

\(^2\) Fast = grill temperature of 249-260°C; Slow = grill temperature of 149°C.

\(^3\) INF = *M. infraspinatus*; TRI = *M. triceps brachii*; REC = *M. rectus femoris*; VAL = *M. vastus lateralis*

\(^a-m\) LS means for an effect without a common superscript differ \((P < 0.05)\)
Table 8. Sensory off-flavor intensity scores\(^1\) from *M. infraspinatus, M. triceps brachii, M. rectus femoris, and M. vastus lateralis*

<table>
<thead>
<tr>
<th>Muscle(^2)</th>
<th>0 h Holding Time</th>
<th>1 h Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fast Cook(^3)</td>
<td>Slow Cook(^3)</td>
</tr>
<tr>
<td>INF</td>
<td>5.83(^{c,d})</td>
<td>5.62(^{c,d})</td>
</tr>
<tr>
<td>TRI</td>
<td>4.86(^{b})</td>
<td>5.82(^{c,d})</td>
</tr>
<tr>
<td>REC</td>
<td>5.70(^{c,d})</td>
<td>5.75(^{c,d})</td>
</tr>
<tr>
<td>VAL</td>
<td>4.28(^a)</td>
<td>5.65(^{c,d})</td>
</tr>
</tbody>
</table>

\(^1\) Off-flavor intensity scores: 8 = no off-flavor, 7 = trace off-flavor, 6 = slight off-flavor, 5 = small off-flavor, 4 = modest off-flavor, 3 = moderately off-flavor, 2 = very off-flavor, 1 = extreme off-flavor

\(^2\) INF = *M. infraspinatus*; TRI = *M. triceps brachii*; REC = *M. rectus femoris*; VAL = *M. vastus lateralis*

\(^3\) Fast = grill temperature of 249-260°C; Slow = grill temperature of 149°C.

\(^4\) SE = 0.36

\(^a-d\) LS means without a common superscript differ (*P* < 0.05)
Table 9. Percentage of panelists denoting specific off-flavors for *M. teres major*, *M. vastus intermedius*, and *M. vastus medialis* at different holding times

<table>
<thead>
<tr>
<th>Effects</th>
<th>Liver-like</th>
<th>Metallic</th>
<th>Sour</th>
<th>Charred</th>
<th>Oxidized</th>
<th>Rancid</th>
<th>Fatty</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>TER</td>
<td>24.3</td>
<td>14.9</td>
<td>38.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.6</td>
<td>7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>VAI</td>
<td>33.9</td>
<td>13.9</td>
<td>27.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.1</td>
<td>22.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>22.5</td>
<td>17.22</td>
<td>43.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.1</td>
<td>8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>8.2</td>
<td>3.0</td>
<td>4.0</td>
<td>4.8</td>
<td>3.0</td>
<td>2.2</td>
<td>2.6</td>
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<td>0.0203</td>
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<td>0.0008</td>
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<tr>
<td>0</td>
<td>23.7</td>
<td>16.1</td>
<td>34.9</td>
<td>28.7</td>
<td>12.9</td>
<td>7.6</td>
<td>7.4</td>
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<tr>
<td>1</td>
<td>30.2</td>
<td>14.6</td>
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<td>6.1</td>
<td></td>
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<tr>
<td>SE</td>
<td>7.5</td>
<td>2.6</td>
<td>3.4</td>
<td>3.9</td>
<td>2.5</td>
<td>1.7</td>
<td>1.9</td>
<td></td>
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<tr>
<td><strong>Muscle x Hold Time (h)</strong></td>
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<tr>
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<tr>
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<tr>
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</tr>
<tr>
<td>SE</td>
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<td></td>
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</tr>
<tr>
<td><em>P</em>-value</td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>TER= *M. teres major*; VAI= *M. vastus intermedius*; VAM= *M. vastus medialis*

<sup>a</sup>-<sup>c</sup> LS Means without a common superscript differ (*P* < 0.05)
Table 10. Percentage of panelists denoting specific off-flavors for *M. infraspinatus*, *M. triceps brachii*, *M. rectus femoris*, and *M. vastus lateralis* at different holding times and cooking rates.

<table>
<thead>
<tr>
<th>Effects</th>
<th>Liver-like</th>
<th>Metallic</th>
<th>Sour</th>
<th>Charred</th>
<th>Oxidized</th>
<th>Rancid</th>
<th>Fatty</th>
<th>Other</th>
</tr>
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<tbody>
<tr>
<td><strong>Muscle</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INF</td>
<td>16.9</td>
<td>17.1</td>
<td>23.5</td>
<td>0.6</td>
<td>3.1</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRI</td>
<td>19.1</td>
<td>39.4</td>
<td>23.7</td>
<td>15.7</td>
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<td>4.5</td>
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</tr>
<tr>
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<td>19.0</td>
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<td>12.9</td>
<td>1.5</td>
<td>3.5</td>
<td></td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>VAL</td>
<td>16.9</td>
<td>37.0</td>
<td>31.5</td>
<td>20.7</td>
<td>7.6</td>
<td></td>
<td>2.8</td>
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</tr>
<tr>
<td>SE</td>
<td>7.1</td>
<td>4.6</td>
<td>3.9</td>
<td>1.6</td>
<td>1.3</td>
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<tr>
<td><strong>P-value</strong></td>
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<td><strong>Hold Time (h)</strong></td>
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<td><strong>P-value</strong></td>
<td>0.6464</td>
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<td>4.4</td>
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<td>9.2</td>
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<td>4.1</td>
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<td>6.9</td>
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INF = *M. infraspinatus*; TRI = *M. triceps brachii*; REC = *M. rectus femoris*; VAL = *M. vastus lateralis*

1 Fast = grill temperature of 249-260°C; Slow = grill temperature of 149°C.

2 LS means without a common superscript differ *(P < 0.05).*
Protocol for determining volatile compound differences between liver-like and normal beef samples using gas chromatography

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ABSTRACT

A gas chromatography (GC) protocol to identify differences in liver-like and normal beef samples was developed. Samples of comparable composition were chosen based on descriptions by panelists as having liver-like off-flavor or having no off-flavors (normal). Five grams of raw powdered samples from *M. rectus femoris* and 100 mL of distilled water were flushed with nitrogen to allow odors to be removed from the sample and escape out through the tube. The volatile compounds being removed were smelled, and time periods were recorded. Two time periods had undesirable smells for liver-like samples not found in normal samples. Volatile compounds were trapped in polymer columns attached to the outlet of the gas dispersion flask for 0-5 and 5-10 min. The columns were flushed with ether (2 mL) and stored in glass vials at -20°C. Ether samples were run with GC oven conditions starting at 35°C, raising 2°C/min until 200°C, and held for ten min. Normal samples demonstrated six peaks not present in liver-like samples, while liver-like samples showed three peaks not present in normal samples. Eight peaks differed in intensity in the two sample types. There are volatile compound differences in the two sample types.

Keywords: Beef, volatile compounds, gas chromatography
1. Introduction

Flavor plays an important role in palatability of beef. Traditionally, the meaty flavor is said to be made up of water soluble compounds in muscle tissue as well as sulfur-containing compounds while fat helps produce the species flavor and aroma when heated which was introduced as early as the 1960s (Hornstein & Crowe, 1960; Hornstein, Crowe, & Sulzbacher, 1960). During thermal processing, the Maillard and Strecker degradation reactions form products that also contribute to cooked beef flavor.

One of the most common forms of off-flavors in meat can develop from lipid oxidation when the double bonds on the fat molecules oxidize. However, off-flavors can be caused by compound changes in the muscle due to animal diets (Vasta and Priolo, 2006) or microbial spoilage.

Miller (2001) adapted Johnson and Civille (1986) lexicon to define the descriptor for livery/organy as the aromatic associated with beef liver and/or kidney. Miller (2001) noted that the livery flavor appears to be bipolar, meaning an individual either likes the flavor or does not, but a low concentration of the compounds responsible for the flavor is easily recognizable.

Miller (2002) reviewed hypotheses about the potential cause of livery flavor in beef such as higher levels of myoglobin, higher pH, improper exsanguination, lipid oxidation, and higher degree of doneness. Yancey et al. (2006) saw an increase in liver flavor of the M. gluteus medius and a decrease in beef flavor as iron increased. Yancey et al. (2006) also found low correlations between 16-18 carbon chain fatty acids and liver flavor.
James and Calkins (2005) demonstrated beef off-flavor intensity, including liver-like off-flavors, was reduced when steaks from beef chuck and round muscles were slow cooked (149°C grill temperature) or held for 1 h in a heated cyclotherm. Undesirable smell was also observed during cooking in the samples that were rated as liver-like. Further study revealed an animal effect for the off-flavors in the muscles tested from the chuck and the round; thus when one muscle was rated as off-flavored, other muscles from the same animal was also rated as off-flavored (Meisinger, James, & Calkins, 2006).

Some studies have been conducted to investigate possible volatile compounds that might be responsible for the liver-like flavor, but used cooked beef (Gorraiz, Beriain, Chasco, & Insausti, 2002; Yancey et al., 2006). Many aromatics, including liver-like, develop during the thermal process so these studies would not have identified volatiles produced during the early stages of heating. Therefore, the objective of this research was to develop a procedure to identify differences in volatile compounds from uncooked beef rated as normal or liver-like in flavor.

2. Methods and materials

2.1 Sample preparation

A previous study (James and Calkins, 2005) identified off-flavored and normal steaks from *M. infraspinatus, M. teres major, M. triceps brachii* long head, *M. rectus femoris, M. vastus lateralis, M. vastus medialis*, and *M. vastus intermedius*. Samples rated as five or above on an 8-point hedonic scale were considered normal. Samples that were rated as off-flavored (below five on an 8-point scale) and identified as being liver-like by a majority of the panelists were used in the initial compound collection study.
Remaining uncooked steaks from one normal and one liver-like *M. rectus femoris* from USDA Select carcasses were homogenized by slicing the steak into smaller pieces, freezing the steak in liquid nitrogen, and pulverizing with a Waring blender (Waring Products Division, New Hartford, CT). Samples were maintained in a -80°C freezer until analyzed. To conserve samples, the final protocol was developed with the *M. rectus femoris* as the other samples ran too low to have a complete comparison between the muscles.

### 2.2 Collection of Volatile Compounds

Five grams of raw powdered meat samples from the *M. infraspinatus, M. teres major, M. triceps brachii* long head, *M. rectus femoris, M. vastus lateralis, M. vastus medialis*, and *M. vastus intermedius* and 100 mL of distilled water were mixed in a glass gas dispersion flask (Figure 1) and flushed with nitrogen gas in a 40°C water bath. Times for the release of off-odors were collected using nitrogen gas flow rates of 54.5 mL/min or 150 mL/min. For collection, the 150 mL/min nitrogen gas flow rate was used with the *M. rectus femoris*. Volatile compounds were trapped in a packed porous polymer (20331 Porapak Q 80-100 mesh, matrix; SUPELCO, Bellefonte, PA) column, pre-washed with ethyl ether, attached to the outlet of the gas dispersion flask for 0-5 min. A second column was attached for 5-10 min. The ends of all columns were quickly wrapped with parafilm after removing from the glass gas dispersion container and stored in a container with desiccant to ensure no additional components were absorbed.

The columns were flushed with ether (2 mL); extracted samples were captured and stored in a glass vial at -20°C for no more than 1 d.
2.3 Gas chromatography

Ether extracts were splitlessly injected (1 μL) in a gas chromatograph (GC; Hewlett-Packard 6890 Series- GC System, Agilent Technologies, Santa Clara, CA) with oven conditions starting at 35°C, raising 2°C/min until 200°C was reached, and held for ten minutes for a 92.50 min run time. A 30 m, 5% phenyl-95% methylpolysiloxane GC column (Alltech Capillary Column ECTM-5, Alltech Associates, Inc, Deerfield, IL) with an inner diameter of 0.32 mm and film thickness of 0.25 μm was used. Gas flow rate was 24.7 mL/min, and the injection and FID temperature were 250°C.

After the initial protocol was developed, an attempt to capture the compounds coming off the GC was carried out to try to capture just the undesirable smells. The FID was turned off and an olfactory device was made and attached to the detector port. As compounds came off the column, an individual would take note of the smell and time with the idea that when peaks of off-flavored smells came off, they could be captured in liquid nitrogen and taken to a mass spectrometer for identification.

2.4 Mass spectrometry

A portion of each ether extracted sample was sent for identification of compounds by mass spectrometry. The samples were injected with a 50:50 split into a GC-Mass Spectrometer (MS) system (Hewlett-Packard GC 6890, Agilent Technologies, Santa Clara, CA; Hewlett Packard MS 5973, Agilent Technologies, Santa Clara, CA). The column (Hewlett-Packard 5MS, Agilent Technologies, Santa Clara, CA) used was 30 m in length with 0.25 mm ID and 0.25 μm film thickness. Identification was made using the NIST/EPA/NIH Mass Spectral Library (Version 2.0a, build July 1, 2002).
3. Results and Discussion

3.1 Collection of volatile compounds

Table 1 shows the results of the different nitrogen gas flow rates. In the off-flavored samples when 54.5 mL/min nitrogen gas flow was used, an undesirable smell started at 1.45 min, peaked at 3.00 min, and faded back to a normal beef smell by 4.15 min. The undesirable smell returned at 6.30 min and dissipated by 7.30 min. Using 150 mL/min nitrogen gas flow rate, the undesirable smell started at 0.36 min and ended at 2.40 min. The odor returned again between 5.20-6.00 min. Interestingly, the normal samples never had an undesirable smell, just a regular beefy aroma. Therefore, time periods collected were 0-5 min and 5-10 min. After initial test runs, it was determined the 5-10 min period gave almost the same results from the chromatograms as the 0-5 min period, but with lower intensities, so the remaining research was conducted on the 0-5 min collection period.

3.2 GC results

Figure 2 shows representative chromatograms from normal and off-flavored samples. The off-flavored samples contained three peaks that were not present in the normal samples while the normal samples had six peaks that were not present in the off-flavored samples (Table 2). Furthermore, concentration differences of several other compounds were seen between the two types of samples. The normal samples showed five compounds with large differences in the concentration as well as two compounds with slight differences. The off-flavored samples had one compound with a slight increase in concentration over the normal sample.
3.3 MS results

Figure 3 shows the chromatograms from the GC-MS analysis of the normal and off-flavored samples. While visual differences could be seen in the chromatograms, many of the peaks remained unidentified by the MS. The peak seen in the off-flavored sample and not in the normal sample before five min might be a hydrogen sulfur-based compound. It is possible the peaks from 16-24 min were either glycine or glycerol isomers. The two major peaks at approximately 15 and 30 minutes were not different between the two samples types and were dimethylbenzene and dimethylbenzene with carboxyl group, respectively.

4. Conclusions

There are differences in the off-flavored and normal samples as shown by the GC and GC-MS when volatile compounds are extracted by this novel procedure. However, to have better identification of compounds by the MS several recommendations were made and tested. 1) Use ethyl acetate instead of ethyl ether because of the higher melting point. This would allow the early compounds to be identified where the ethyl ether was coming off. Using ethyl acetate was not a viable option unless all GC conditions were changed as well as making changes to the porous polymer pack column as the ethyl acetate did not have the affinity for the compounds like ethyl ether. 2) Obtain a more concentrated sample for the GC-MS system. The volatile compounds were caught in the same porous polymer column numerous times for the same sample during the 0-5 min collection time. Fresh sample and new dH2O were used each time. After repeating for the 6th time, smells started to escape the column so the samples were only concentrated
five times. Because of the split injection, the concentrated samples still did not allow for identification of some of the unknown compounds. 3) Use more sample in the initial step. This was not a practical alternative as sample amount was low.

Using the method described previously, one would be able to determine visual differences between off-flavored and normal samples with gas chromatography. Differences between muscles would also be able to be seen. Another method should be used for identification of compounds by MS. Once the compounds are identified, the gas chromatography method explored in this study could be used with standards to confirm differences between samples without the added expense of a MS.

Acknowledgements

1 A contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE 68583.

2 This project was funded by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen’s Beef Board and state beef councils by the National Cattlemen’s Beef Association.

3 A note of thanks to Daniel Snow, PhD and David Cassada, PhD with the UNL Water Science Lab for their assistance and suggestions with the mass spectrometry work.
References


Figure 1. Schematic of the gas dispersion container
Figure 2. Gas chromatograms from off-flavored and normal *M. rectus femoris* samples. The arrows are pointing to peaks or groups of peaks that are present in one sample and not in the other or are in different concentrations in the two sample types.
Figure 3. Chromatograms from gas chromatography-mass spectrometry from off-flavored and normal *M. rectus femoris*. 
Table 1. Times of undesirable smells coming out of the gas dispersion container using different nitrogen gas flow rates for samples rated as off-flavored.

<table>
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<th>Nitrogen Gas Flow Rate</th>
<th>Time odor starts (min)</th>
<th>Time odor ends (min)</th>
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<tr>
<td>54.5 mL/min</td>
<td>1.45</td>
<td>4.15</td>
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<td>6.30</td>
<td>7.30</td>
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<tr>
<td>150 mL/min</td>
<td>0.36</td>
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<td>5.20</td>
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Table 2. Retention times and areas for off-flavored and normal *M. rectus femoris* samples

<table>
<thead>
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<th>Area of Normal Sample</th>
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<td>3451</td>
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<td>2.165/ -</td>
<td>3953</td>
<td>2738</td>
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<td>-/2.310</td>
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<td>32279</td>
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<td>2.371/2.379</td>
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Identification of Volatile Compounds in Beef Round and Chuck Muscles

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ABSTRACT

Volatile off-flavor compounds are present in beef. Using purge and trap gas chromatography and a mass spectrometry system, some volatile compounds were shown to have different concentrations in normal-flavored beef, compared to samples with liver-like off-flavor. Most of the compounds, like pentanol, hexanal, hexanol, 1-octen-3-ol, and nonanal, are associated with lipid oxidation. The compounds β-pinene, 1-octen-3-ol, and 2,4-decadienal were in higher concentration in the liver-like samples in all muscles tested. Several, small, unidentified peaks also differed between samples. Comparison of normal muscles showed small differences in volatile profiles that might help explain differences in flavor profiles among muscles. Determination of the possible origins of these compounds may improve the quality and consistency of beef products.

Keywords: Purge and Trap Mass Spectrometry, Volatile Compounds, Beef
1. Introduction

Flavor is an important factor in beef palatability. Hornstein, Crowe, and Sulzbacher (1960) found the precursors of cooked beef flavor to be water soluble and low molecular weight compounds in the lean fraction of meat (Hornstein and Crowe, 1960). Meat flavor is typically developed through reactions between amino acids and carbohydrates in addition to the flavor created by the fatty acid profile.

Numerous studies have investigated factors that may play a role in the beef flavor profile such as sex (Gorraiz, Beriain, Chasco, & Insausti, 2002), breed (Gorraiz et al., 2002; Monson, Sierra, & Sanudo, 2005), diet (Meyer, Thomas, Buckley, & Cole, 1960; Brown, Melton, Riemann, & Backus, 1979; Melton, 1990; Schnell, Belk, Tatum, Miller, & Smith, 1997), muscle (Carmack, Kastner, Dikeman, Schwenke, Garcia Zepeda, 1995; Yancey, Dikeman, Hachmeister, Chambers, & Milliken, 2005; Meisinger, James, & Calkins, 2006), aging time (Gorraiz et al., 2002; Monson et al. 2005), storage condition (Brown et al., 1979), and lipid degradation (Kim, Nam, & Ahn, 2002; Campo, Nute, Hughes, Enser, Wood, & Richardson, 2006).

Renewed interest in determining the cause of the liver-like off-flavor in beef has lead to many hypotheses, but no firm idea as to a plausible source of the unpleasant odor. Research has been conducted to investigate the effects of quality grade (Yancey, 2002), aging (Calkins, 2002b; Gorraiz et al., 2002; Yancey, 2002), marination (Streff, Wulf, & Maddock, 2003), degree-of-doneness (Adhikari, Keene, Heymann, & Lorenzen, 2004; Calkins, 2002a; Streff et al., 2003), and cooking methods (Adhikari et al., 2004; Miller, 2001) on the occurrence of this off-flavor. Gorraiz et al. (2002) and Yancey (2002)
investigated cooked beef for volatile compounds and found several compounds that appeared to be tied to the liver-like flavor.

Hodgen, Cuppett, & Calkins (2006) discovered volatile compounds from liver-like muscles were released at temperatures as low as 40°C. At this low temperature, many of the compounds that develop due to Maillard browning and thermally induced lipid oxidation are not produced in the sample; in addition artifact compounds that are produced at higher temperatures are eliminated (Ahn, Jo, & Olson, 1999). Utilizing raw samples rather than cooked samples when investigating this off-flavor might allow the precursor compounds to be identified more easily.

Therefore, to minimize undesirable volatile off-flavors, an understanding of the compounds being produced in off-flavored samples compared to normal samples is worthwhile. The primary objective of this research was to identify differences in volatile compounds between raw, uncooked steaks with liver–like off–flavors and normal muscles. This study also investigated the aromatic compound differences among muscles designated as normal in flavor.

2. Methods and materials

The *M. infraspinatus* (Flat Iron; INF), *M. triceps brachii* (Clod Heart; TRI), *M. rectus femoris* (Knuckle Center; REC), *M. vastus lateralis* (Knuckle Side; VAL), and *M. vastus intermedius* (Knuckle Bottom; VAI) were evaluated. These muscles from USDA Select carcasses were identified as ‘liver–like’ or ‘normal’ by a trained taste panel with ‘normal’ classification having an off–flavor rating of five or above on an 8–point scale (James and Calkins, 2006). Liver-like samples used had a rating below five on the 8-point scale for off-flavor intensity with at least half the panelists identifying liver-like as a
specific off-flavor in the sample. Normal INF were not available at the time of testing, but the four other muscles had two to three liver-like samples and at least two normal samples tested. Five grams of raw, frozen, pulverized sample were weighed into 50 mL glass injection vials and maintained frozen (-80°C) until run (< 2 d). Frozen samples were placed in an autosampler (O-I-Analytical Water/Soil Autosampler, Model 4552, College Station, TX) and 10 mL of distilled water were added to each vial prior to its run. The purge and trap system (O-I-Analytical Eclipse, Model 4660, College Station, TX) heated the sample to 40 or 80°C and flushed the sample with purified helium gas to allow the volatiles to separate from the sample. Purge time was 11 min at 30°C in a Trap10 trap (O-I-Analytical, College Station, TX). After purging, the volatiles were desorbed 4 min at 190°C. The subsequent run on the GC/MS (Agilent Technologies 6890N Network GC system) used a 30 m, 0.25 mm ID, and 0.25 μm film thickness column. The volatile compounds were held at 40°C for 4 min followed by an 8°C/min ramp to 250°C and held for 10 min. Compound masses were then determined with a mass spectrometer (Agilent Technologies 5973 inert Mass Selective Detector) and identified by the database (NIST/EPA/NIH Mass Spectral Library, Version 2.0a, Scientific Instrument Services, Ringoes, NJ).

Relative comparisons of volatile compounds between liver-like and normal samples were made. Differences among two normal samples from one muscle in the chuck (M. triceps brachii) and normal samples obtained from three muscles from the knuckle (M. rectus femoris, M. vastus intermedius, and M. vastus medialis) were also studied.
3. Results and discussion

The samples heated to 40°C yielded similar peaks to the samples heated to 80°C. However, because the samples were raw, the concentration was not high enough in the samples heated to 40°C for positive identification of compounds by the mass spectrometer database. Therefore, the results presented in this paper are based on the compounds identified when the samples were heated to 80°C in the purge and trap system.

Sulfur compounds, like thiols and pyrazines, were not seen in this study since these compounds would not have developed in raw samples. Continued efforts are needed to investigate this aspect of the liver-like off-flavor in beef muscles.

3.1 Comparison of normal and liver-like samples

Thirty-eight to 74 volatile compounds were present in the samples, with normal TRI having the least number of compounds and liver-like VAI having the most. Differences in the presence and concentration of compounds were noted between liver-like and normal samples. Several small, unidentified peaks were absent in liver-like samples, but present in the normal. Approximately four peaks were present in the liver-like samples, but absent in the normal samples. When the concentrations of the compounds were different, the normal samples, in most cases, had lower concentrations (Table 1). Most of the compounds found in greater amounts in the liver-like samples are associated with lipid oxidation, such as pentanal, hexanal, hexanol, 1-octen-3-ol, and nonanal. Hexanal has been suggested as an indicator of deterioration in meat flavor since its concentration increases much faster than other aldehydes, but specific evaluation times
must be set as hexanal levels have a spike so there are two different points of increase during the storage of cooked meat (Shahidi & Pegg, 1994).

When the different compounds are broken into their respective classes, the majority of compounds are aldehydes (Table 2). These aldehydes have been found in other studies to contribute to the volatile profile of other meat products (Elmore & Mottram, 1997; Nam, Hur, Ismail, & Ahn, 2001; Gorraiz et al., 2002; Muriel, Antequera, Petron, Andres, & Ruiz, 2004; Liu, Xu, Ouyang, & Zhou, 2006) and usually have a significant impact on aroma because of their low detection thresholds (Fazzalari, 1978). When beef animals were fed increasing levels of polyunsaturated fatty acids (PUFA) in their diet, the level of lipid oxidation aldehydes were also in higher concentration (Elmore, Mottram, Enser, & Wood, 1999). Thermal degradation of oleic and linoleic acid seem to be induced by increases in dietary PUFA. Unlike most furans, the furan (pentylfuran) found in the samples can be detected at very low thresholds (4 ppb) (Elmore et al., 1999). The hydrocarbons listed generally come from lipid oxidation, although Ruiz, Ventanas, & Cava (2001) hypothesized some of these compounds may come from animal feed and may bypass rumen biohydrogenation. Ketones and alcohols also contributed to the aromatic profile of beef. The VAI had one epoxide, a three-membered cyclic ether.

The compounds, β-pinene and 1-octen-3-ol, were in higher concentration in the liver-like samples in all muscles tested. As mentioned previously, 1-octen-3-ol is related to lipid oxidation from linoleic acid (Muriel et al., 2004). Beta-pinene is an oxidation product of limonene (a common citrus aromatic terpene) as well as a terpene found in pine trees and their berries, in addition to many plants such as creosote, cedar, and
2,4-decadienal were present in the liver-like samples, but were in lower concentration or not present (TRI) in normal samples. The compound 2,4-decadienal is usually associated with fatty or oily aroma, and is an autoxidation product of linoleic acid that has an extremely low detection threshold (Chen & Ho, 1998).

Im, Hayakawa, & Kurata (2004) identified compounds in porcine liver that were induced by iron oxidation. These compounds helped impart the undesirable metallic, weak metallic, mushroom-like, cardboardy, and fatty off-flavors and odors in porcine liver, and liver-like off-flavors were highly correlated to the perception of fishy and metallic off-flavors. Four of the five compounds implicated, 1-octen-3-one, hexanol, 1-octen-3-ol, (E)-2-nonenal, and (E,E)-2,4-decadienal, were found in higher concentration in the off-flavored muscles in this study. Visual determination of concentration differences between normal and liver-like muscles revealed hexanol, 1-octen-3-ol, and 2,4-decadienal had the same trend as seen by Im et al. (2004). Yancey et al. (2006) identified 16 compounds that were different between liver-like and normal cooked samples with many of the compounds being oxidation byproducts. This study found five of those same compounds having different concentrations, although most of the compounds Yancey et al. (2006) identified were not different for the liver-like and normal samples in this study. Because of the small sample size in this study and the low concentrations of the additional compounds, there may be some differences that were not revealed in this study since raw muscle was used and the compounds would not be as concentrated as compounds in cooked samples. It is important to note some compounds
have extremely low thresholds so even a small, but significant, change in concentration can alter the flavor profile from acceptable to unacceptable.

One hypothesis for the origin of the lipid derived off-flavors in meat is the diet of animals. Different grasses have been shown to create less desirable meat flavor because of changes in lipid deposition and fatty acid composition (Melton, 1983). Grass-based animal diets produced meat with higher fishy and bloody ratings and lower overall flavor liking scores compared to concentrate-fed animals partially due to increased 18:1trans isomers and CLA cis-9, trans-11, the most prominent CLA isomer (Neurnberg et al., 2005). Furthermore, CLA trans-7, cis-9 is the second most abundant CLA isomer in muscle tissue from concentrate fed animals while CLA trans-11, cis-13 is the second most abundant CLA in muscle tissue when beef animals are finished on grass diets (Dannenberger et al., 2004). These changes are likely to occur because of the decrease in Δ⁹-desaturase activity in grass-fed animals (Dannenberger et al., 2004). This elongase, in conjunction with trans vaccenic acid, is responsible for synthesis of CLA cis-9, trans-11. By disrupting the elongase activity, flavor changes might occur because of the unused trans vaccenic acid, a fatty acid implicated in off-flavors (Camfield, Brown, Lewis, Rakes, & Johnson, 1997), and lower levels CLA cis-9, trans-11 (Dannenberger, Nuernberg, Scollan, Steinhart, & Ender, 2005).

3.2 Comparison of volatiles from selected chuck and round muscles

Twenty-eight compounds were identified in all four normal muscles (Table 3 & 4). The TRI did not have any unique compounds from the muscles in the knuckle. The VAL had two unique aromatic compounds in its volatile profile. This muscle had a second isomer of 1-hexanol at 8.25 min in the run and 2-nonanone at 13.52 min in the
run. Both of these compounds were in low concentrations relative to other compounds in the sample. The VAI possessed four compounds that were unique to that muscle, with one of the compounds being unknown. Piperazine, (Z)-3-octene, and 1,3-bis(1,1-dimethylethyl)benzene made up the other three unique aromatics in the VAI. The relative quantity of piperazine and (Z)-3-octene in the VAI is significant compared to the other compounds that make up the volatile profile of the VAI. These compounds may help explain why the VAI is perceived as having moderately intense flavor (Brickler, 2000; Jones, Calkins, Johnson, Carpenter, Guru, & Ashu, 2004). The REC had the most unique compounds with ten. These include sec-butanamine, 5-amino-1-pentanol, N-methyl-1,3-propanediamine, (E)-2-hexen-1-ol, 3-octen-2-one, octadecanal, undec-4-enal, 2,4-nonadienal, and two unknowns. Additionally, the REC had higher concentrations of many of the compounds in comparison to the other three muscles. The beef myology website reports the REC has slightly intense flavor whereas the VAL, VAI, and TRI have moderately intense flavor (Jones et al., 2004). Apparently, the additional compounds tone down the REC flavor so it is not perceived as intense or off-flavored as other muscles in the chuck and round (Brickler, 2000).

Interestingly, mass spectrometry also revealed that the three muscles from the knuckle have ten additional compounds that are not found in the TRI. These findings may explain why the TRI was found to have higher off-flavor ratings than the other three muscles by Brickler (2000). The REC and VAI also have eight compounds in common that the TRI and VAL never exhibit, while the REC and VAL have four compounds that the TRI and VAI do not possess.
These different combinations of volatiles may help explain the slight difference in the perceived flavor profile. The raw samples used in this study originated from a study looking at cooking rate and holding time effects on off-flavor (James, & Calkins, 2006). One can begin to gain an understanding of how the unique compounds from each muscle helps with the overall flavor characteristics in each muscle. The unique compounds of VAI create a flavor profile that has higher liver-like, metallic, and rancid characteristics than the TRI, REC, and VAL (James, & Calkins, 2006). All the volatile compounds in the REC may help reduce the sour taste and oxidized, rancid, and charred aromas (James, & Calkins, 2006) when compared to the other muscles in this study. While the VAL only had two different compounds from the other muscles, the combination of these compounds helped reduce the perceived liver-like characteristic in these samples; although Meisinger et al. (2006) found no difference in liver-like between the REC, TRI, and VAL.

Further research with more muscles and larger sample size could help further characterize muscles by explaining differences in flavor among the muscles. This information might help with developing recipes by enhancing the natural flavor of each muscle.

4. Conclusion

Differences were observed in volatile compounds between liver–like and normal beef muscles from the chuck and round. A combination of aromatic compounds, not a single compound, appears to contribute to the undesirable flavor. Research to determine the possible origins of these compounds in beef, with special interest in animal diets high
in PUFA and oxidative reactions with oleic and linoleic fatty acids, is necessary to ensure that quality and consistency of meat from these muscles is acceptable to consumers.

Acknowledgements

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2This project was funded in part by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen’s Beef Board and state beef councils by the National Cattlemen’s Beef Association.

3A special thanks is extended to Daniel Snow and David Cassada in the UNL Water Resources Department for their assistance with the mass spectrometry work.
References


Yancey II, E. J. (2002) Determination of factors causing livery flavor in steaks from the beef loin and chuck. *PhD Dissertation*. Kansas State University, Manhattan, KS.


Table 1. Compound concentration differences between the liver-like and normal-flavored beef muscles

<table>
<thead>
<tr>
<th>Compoundab</th>
<th>TRIc</th>
<th>RECc</th>
<th>VALc</th>
<th>VAIc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver-like</td>
<td>Normal</td>
<td>Liver-like</td>
<td>Normal</td>
</tr>
<tr>
<td>2,3-Dimethyl Oxirane</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Pentanal</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Heptanol</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Hexanal</td>
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<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Hexanol</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Heptanal</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>↑</td>
<td>↓</td>
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<td>↓</td>
</tr>
<tr>
<td>1-Octen-3-ol</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>2-Methyl-3-Octanone or n-Caproic Acid, Vinyl Ester</td>
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<td>↓</td>
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<td>↓</td>
<td>↑</td>
<td>↓</td>
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<td>Octanal</td>
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<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Pinene</td>
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<td>↓</td>
</tr>
<tr>
<td>2-Octenol</td>
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<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
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<td>1-Octanol</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Nonanal</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Hydroxymandelic Acid</td>
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<td>↓</td>
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<td></td>
</tr>
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<td>2-Nonenal</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,3-bis (1,1-Dimethylethyl) Benzene</td>
<td>↑</td>
<td>↓</td>
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<td></td>
</tr>
<tr>
<td>2,4-Decadienal</td>
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<td>NP</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>2,4-Decadienal</td>
<td>↑</td>
<td>NP</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

a Compounds listed revealed visual concentration differences in chromatograms
b ↑ indicates that a higher concentration of the compound was found in that type of sample; ↓ indicates that a lower concentration of the compound was found in that type of sample; NP=not present
c TRI = M. triceps brachii, REC = M. rectus femoris, VAL = M. vastus lateralis, and VAI = M. vastus intermedius.
Table 2. Classification of volatile compounds identified by mass spectrometry

<table>
<thead>
<tr>
<th>Aldehydes</th>
<th>Ketones</th>
<th>Alcohols</th>
<th>Furans</th>
</tr>
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<td>2-Heptanone</td>
<td>Heptanol</td>
<td>2-Pentylfuran</td>
</tr>
<tr>
<td>Hexanal</td>
<td>2-Methyl-3-Octanone(^a)</td>
<td>Hexanol</td>
<td></td>
</tr>
<tr>
<td>Heptanal</td>
<td></td>
<td>1-Octen-3-ol</td>
<td></td>
</tr>
<tr>
<td>Octanal</td>
<td></td>
<td>1-Octanol</td>
<td></td>
</tr>
<tr>
<td>2-Octenal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonanal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Nonenal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-Decadienal, (E)(E)</td>
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<td></td>
</tr>
<tr>
<td>2,4-Decadienal</td>
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<tr>
<td><strong>Hydrocarbons</strong></td>
<td><strong>Epoxides</strong></td>
<td><strong>Fatty Acid</strong></td>
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</tr>
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<td>2,3-Dimethyloxirane</td>
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<tr>
<td>1,3-bis(1,1-Dimethyl)Benzene</td>
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</table>

\(^a\) These two compounds had the same probability of being the compound found at 11.01 min retention time (i.e. only one was actually present).
Table 3. Compound concentration differences for 0-11 min between normal-flavored beef muscles

<table>
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<th>Retention Time(^b)</th>
<th>Compound(^c,d)</th>
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<th>REC</th>
<th>VAL</th>
<th>VAI</th>
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<td></td>
<td></td>
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<td>sec-Butanamine</td>
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<td>1-Octen-3-ol</td>
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</table>

\(^a\) TRI = *M. triceps brachii*, REC = *M. rectus femoris*, VAL = *M. vastus lateralis*, and VAI = *M. vastus intermedius*.

\(^b\) Retention time was obtained from GC-MS report for each sample.

\(^c\) Compounds listed were matches found in the mass spectrometry database. UNK = unknown.

\(^d\) ↑ indicates that a higher concentration of the compound was found in that type of sample; ↓ indicates that a lower concentration of the compound was found in that type of sample; X indicates the compound was present and/or fell in between higher and lower than ones listed with an arrow.
Table 4. Compound concentration differences for 11-22 min between normal-flavored beef muscles\(^a\)

<table>
<thead>
<tr>
<th>Retention Time(^b)</th>
<th>Compound(^c,d)</th>
<th>TRI</th>
<th>REC</th>
<th>VAL</th>
<th>VAI</th>
</tr>
</thead>
<tbody>
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<td>11.01</td>
<td>n-Capric acid vinyl ester or 3-Octanone, 2-methyl</td>
<td>X</td>
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<td>X</td>
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<tr>
<td>11.19</td>
<td>Furan, 2-pentyl</td>
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<td>X</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
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<td>↓</td>
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<td>X</td>
<td>↓</td>
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<td>12.77</td>
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<td>13.00</td>
<td>2-Octen-1-ol or 1,3-Octadiene</td>
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<tr>
<td>13.05</td>
<td>1-Octanol</td>
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<tr>
<td>13.80</td>
<td>Nonanal</td>
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<td>↑</td>
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<td>14.83</td>
<td>3-Hydroxyxymandelic acid, ethyl ester, di-TMS or Benzaldehyde, 2,4-bis(trimethylsiloxy)-</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>14.99</td>
<td>2-Nonenal or 1-Tetradecene</td>
<td>X</td>
<td>↑</td>
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<td>16.93</td>
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<td>17.03</td>
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<td>X</td>
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<td>18.19</td>
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<td>↑</td>
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<td>18.93</td>
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<tr>
<td>19.51</td>
<td>Tetradecane</td>
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<td>19.71</td>
<td>Oxirane, hexadecyl or UNK</td>
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<td>X</td>
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<td>21.43</td>
<td>UNK</td>
<td>X</td>
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</tbody>
</table>

\(^a\) TRI = *M. triceps brachii*, REC = *M. rectus femoris*, VAL = *M. vastus lateralis*, and VAI = *M. vastus intermedius*.

\(^b\) Retention time was obtained from GC-MS report for each sample.

\(^c\) Compounds listed were matches found in the mass spectrometry database.

\(^d\) ↑ indicates that a higher concentration of the compound was found in that type of sample; ↓ indicates that a lower concentration of the compound was found in that type of sample; X indicates the compound was present and/or fell in between higher and lower than ones listed with an arrow.
Figure 1. Chromatogram from off-flavored M. triceps brachii
Figure 2. Chromatogram from normal M. triceps brachii
Uncooked Beef Muscles with Liver-Like Flavor are Similar in Volatile Compounds to Raw Beef Liver

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ABSTRACT

Concern has been expressed over the descriptive term liver-like to refer to beef muscles with a specific aromatic off-flavor. The objective of this research was to investigate the volatile compound similarities between raw beef liver and muscles rated as being liver-like by a trained sensory panel. The purge and trap mass spectrometry method was used to identify the volatile compounds. Sixteen compounds that are found to be different in liver-like rated muscles from normal flavored muscles were also identified in the raw beef liver sample. Four additional compounds identified in beef liver were identified in both normal and liver-like muscles from the chuck and round. Several other compounds that have similar retention times to unidentified compounds in liver-like samples are present in raw beef liver. Due to the similarities of the volatile profiles between raw beef liver and muscles identified has having a liver-like off-flavor, the terminology ‘liver-like’ is an appropriate descriptor term for the off-flavor in specific cuts from the beef chuck and round.

Keywords: Flavor, Liver, Beef Muscles, Volatile Compounds
1. Introduction

To describe sights, feelings, or tastes, humans have a tendency to try to relate new, unique sensations to something familiar. Belk, Miller, Evans, Lui, & Acuff (1993), Camfield, Brown, Lewis, Rakes, & Johnson (1997), and others have used the term livery to describe an aromatic flavor sometimes associated with meat. Our laboratory and Im, Haya, & Kurata (2004) have used the term liver-like.

Despite a growing frequency of this flavor descriptor, a significant question remains: Does beef with an off-flavor described as liver-like have any common elements to beef liver itself? This is important for several reasons. 1) A study has shown a small amount of this flavor to help develop the desirable flavor of cooked beef (Mandell, Buchanan-Smith, & Campbell, 1998). 2) Some people enjoy the flavor and nutritional benefit of liver. 3) With the export market slowly being opened up to USA beef, there has been a need to promote offal items to the USA public.

Therefore, the objective of this research was to investigate the volatile compounds of liver and compare those compounds to compounds found in meat identified as liver-like.

2. Materials and methods

A pound of raw liver was purchased from a local supermarket, minced, frozen in liquid nitrogen, and pulverized with a Waring blender (Waring Products Division, New Hartford, CT). Two pulverized samples of *M. infraspinatus*, *M. rectus femoris*, *M. triceps brachii*, and *M. vastus lateralis* that had been identified as liver-like during a previous cooking rate and holding time study (James & Calkins, 2006) were used for comparison.
Five grams of raw, pulverized sample were weighed into 50 mL glass injection vials and maintained frozen (-80°C) until run (< 2 d). Samples were placed in an autosampler (O-I-Analytical Water/Soil Autosampler, Model 4552, College Station, TX) and 10 mL of distilled water were added to each vial prior to its run. The purge and trap system (O-I-Analytical Eclipse, Model 4660, College Station, TX) heated the sample to 40 or 80°C and used purified helium as a carrier gas to allow the volatiles to separate (purge) from the sample. Purge time was 11 min at 30°C in a Trap10 trap (O-I-Analytical, College Station, TX). After purging, the volatiles were desorbed four min at 190°C. The subsequent run on the GC/MS (Agilent Technologies 6890N Network GC system) used a 30 m, 0.25 mm ID, and 0.25 μm film thickness column. The volatile compounds were held at 40°C for four min followed by an 8°C/min ramp to 250°C and held for 10 min. Compound masses were then determined with a mass spectrometer (Agilent Technologies 5973 inert Mass Selective Detector) and identified by the database (NIST/EPA/NIH Mass Spectral Library, Version 2.0a).

Relative comparisons of volatile compounds between raw beef liver and raw liver-like beef muscle samples were made.

3. Results and discussion

Volatile compounds found in liver samples when heated to 40°C or 80°C are found in Table 1. Sixteen of the 22 compounds identified as being higher in concentration in muscles rated as liver-like than in normal flavored muscles (Hodgen, Cuppert, & Calkins, 2006), were also identified as volatiles in the raw liver. The compounds 1-octen-3-ol and 2,4-decadienal were two of the three compounds that
were in all the liver-like samples. These two compounds also contributed significantly to the volatile profile of raw liver. Neither α- nor β-pinene were present in the liver samples. There were several unknown compounds in the liver-like samples, but the liver had compounds that were in large enough concentration at the same periods as some of the unknowns in the liver-like samples such as 3-methylbutanal. It would be premature, however, to assume that those are the same compounds.

Im et al. (2004) looked at the relationship of sensory evaluation and gas chromatograph-olfactory to volatile compounds in porcine liver and found the stronger the metallic and fishy notes, the higher the perception of liver-like flavor. Several of the compounds (hexanal; 1-penten-3-ol; heptanal; octanal; hexanal; 2,4-heptadienal; 2-nonenal; 3,5-octadien-2-one; 2,6-nonadienal; 2,4-nondienal; 2,4-decadienal) they discovered were also in the raw beef liver samples. Compounds of particular interest in creating the porcine flavor were 1-octen-3-one (metallic), hexanal (weak metallic), 1-octen-3-ol (mushroom-like), 2-nonenal (cardboard-like), and 2,4-decadienal (fatty oily). All of the compounds except 1-octen-3-one were found in both the raw beef liver samples and the muscles identified as liver-like.

Lorenz, Stern, Flath, Haddon, Tillin, and Teranishi (1983) stated that the lower molecular weight compounds, which consisted mainly of aldehydes, were the most important fraction in the characteristic odor of sheep liver. Pentanal and 3-methylbutyl made up almost 37% of the low molecular weight aldehydes in that study.
Comparison of the results from this study using raw liver with studies using cooked liver yielded similar results with the exception of more Maillard and Strecker degradation compounds brought about because of the cooking process. In addition to the thermally induced compounds, in cooked pork liver, Mussinan and Walradt (1974) identified many of the same compounds found in the beef liver and muscles rated as liver-like including limonene (precursor to α- and β-pinene) and 1-octen-3-ol.

Based on the similar results from the volatile compounds found in raw and cooked muscles rated as liver-like (Hodgen, et al., 2007; Yancey, et al. 2006), the volatile compounds found in raw beef liver, and the data from numerous other liver compound studies, the term liver-like is acceptable terminology for the aromatic off-flavor perceived in some beef muscles.

Acknowledgements

1This paper is a contribution of the University of Nebraska Agricultural Research Division.

2A special thanks is extended to Daniel Snow and David Cassada in the UNL Water Resources Department for their assistance with the mass spectrometry work, and Sherri Pitchie for secretarial support.
References


Table 1. Volatile compounds found in raw beef liver.

<table>
<thead>
<tr>
<th>RT(^a)</th>
<th>Compound</th>
<th>Liver</th>
<th>Liver</th>
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<td>Butanal, 3-methyl</td>
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<td></td>
</tr>
<tr>
<td>3.20</td>
<td>Benzene</td>
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<td>1-Penten-3-ol</td>
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<td>11.67</td>
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<td>Pentanal</td>
<td>X</td>
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<td>10.30</td>
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<td>Benzaldehyde</td>
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<td>1-Octen-3-ol</td>
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<td>2,4-Heptadienal</td>
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\(^a\) RT= retention time
Table 2. Volatile compounds that were in liver and in higher concentration in liver-like muscle samples compared with normal samples

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Compounds</th>
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<td>Pentanal</td>
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<td>1-Pentanol</td>
<td>2-methyl-3-Octanone / n-Caproic Acid</td>
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<td>Octanal</td>
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<tr>
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<td>2-Octenal</td>
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<td>1-Hexanol</td>
<td>2-Nonanone</td>
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</tr>
<tr>
<td>Heptanal</td>
<td>2-Nonenal</td>
</tr>
<tr>
<td>2-Heptenal</td>
<td>2,4-Decadienal</td>
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Validation of the Purge and Trap Mass Spectrometer Results with SPME using M. triceps brachii and Verification of Compounds with M. rectus femoris

J.M. Hodgen\textsuperscript{a}, C. A. Hall III\textsuperscript{b}, S. L. Cuppett\textsuperscript{c}, C. R. Calkins\textsuperscript{a*}

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ABSTRACT

Validation and verification of the purge and trap mass spectrometry method for comparison of normal and liver-like muscle samples were conducted. Another method, solid phase microextraction (SPME), gave very similar results to the purge and trap system. However, some lower molecular weight compounds were identified by SPME that were not seen with the purge and trap while the purge and trap was able to identify more of the total number of compounds. The same trends were seen in terms of concentrations of volatile compounds in normal and liver-like samples. In the verification study, when additional *M. rectus femoris* samples were run with the purge and trap mass spectrometric method, results showed similar compounds to the normal samples run in the initial trial. Toluene and limonene were identified in this study, however. When more liver-like samples are identified by taste panel further evaluation of the method is necessary to verify the results seen in the initial comparison.

Keywords: Validation, Verification, SPME, Purge and Trap Mass Spectrometry
1. Introduction

Solid phase microextraction (SPME) is a quick, solventless assay to separate particulates in a sample. There are several benefits to this method including elimination of steps that can change the properties of volatile compounds and elimination of hazardous solvents. In the early 1990s Dr. Janusz Pawliszyn of the University of Waterloo in Ontario, Canada introduced the method in which a specially-coated, silica fiber was placed on a syringe-like device to extract desired sample volatiles that could be analyzed with gas chromatography (GC) (Arthur, & Pawliszyn, 1990). Further automation and development of the technique has allowed for a wide range of applications with GC, liquid chromatography, mass spectrometry (MS), supercritical fluid chromatography, and capillary electrophoresis.

Purge and trap is a dynamic headspace analysis technique also called direct thermal desorption. A major benefit to this analysis is the reduction of the sample matrix effect. In this system, samples with volatile organic compounds are placed in a purge container and flushed with an inert gas at a constant flow rate for a specific time. Volatile compounds are concentrated into the absorbent trap. When the purging is complete the trap is heated quickly and backflushed with carrier gas to desorb the compounds for analysis in the GC.

Pfannkoch & Whitecavage (2000) compared three direct analyses of volatiles from solid matrices conducted under the same conditions. Static headspace was the least sensitive, but gave adequate information if substantial sample size was available and when there was high water content in the matrix. Solid phase microextraction with the 100 μm polydimethylsiloxane fiber was 10-50 times more sensitive than static headspace, and was useful with high moisture samples. Direct thermal extraction (purge and trap)
was 50-100 times more sensitive than SPME and 500-5000 times more sensitive than static headspace in addition to only needing a small sample size. Two problems arose from direct thermal extraction. With the small sample size, variable results were obtained because of samples that were not homogeneous. This analysis also needs an inlet liner to eliminate water interferences with higher moisture samples.

Validating and verifying methods and results is crucial in the scientific process so the purpose of this research was 1) to compare volatile compounds identified with the purge and trap GC-MS with those found with SPME and 2) run the purge and trap GC-MS method on additional M. rectus femoris samples to compare results with initial findings.

2. Materials and methods

Due to availability of pulverized muscles in which a sensory evaluation had been conducted on a corresponding steak, the same muscle was not used for the validation and verification.

2.1 SPME

Solid phase microextraction was run according to Fernando, Berg, & Grun (2003). Briefly, 0.5 g pulverized M. triceps brachii (n=2 liver-like; n=2 normal) that had been identified by a trained taste panel as normal or liver-like in flavor (James & Calkins, 2006) were weighed into 4 mL vials with 1.0 g of distilled water and vortexed for 10 sec. The samples were incubated at 90°C for 10 min and vortexed for 10 sec. The samples were allowed to absorb onto the filament (57328-U; 50/30 DVB/CAROXEN/PDMS Stable Flex; Supleco) in a 50°C water bath, and desorbed on the gas chromatogram (GC) inlet for 5 min. Injector temperature was 250°C while the detector temperature was
275°C. Oven parameters were 35°C for 5 min, increase 8°C/min to a temperature of 75°C, followed by a ramp of 40°C/min to 200°C and held for 5 min. Hexanal, pentane, and propanal were used as standards while other compounds from the SPME method were identified by mass spectrometry. Relative quantitative comparisons for normal and liver-like samples were only done on hexanal, propanal, and pentane since those compounds were the only standards run. Results were compared to compounds identified in Hodgen, Cuppett, & Calkins (2006).

2.2 Verification of compounds found in the M. rectus femoris with Purge and Trap MS

Six (3 USDA Choice; 3 USDA Select) M. rectus femoris from the innovative selection procedure of Jenschke et al. (2006) were selected to verify the presence of volatile compounds that were used to compile the list of volatile compounds from raw muscle samples (Hodgen, Cuppett, & Calkins, 2006). Two of the USDA Choice and two of the USDA Select had off-flavor intensities above one (although none were > 1.5 on a 15 point scale) with a liver-like rating of 0.5. The two remaining samples had an off-flavor rating of zero and liver-like rating of zero. Five grams of raw, pulverized sample were weighed into 50 mL glass injection vials and maintained frozen (-80°C) until analyzed (< 2 d). Samples were placed in an autosampler (O-I-Analytical Water/Soil Autosampler, Model 4552) and 10 mL of distilled water were added to each vial prior to its analysis. The purge and trap system (O-I-Analytical Eclipse, Model 4660) heated the sample to 80°C to allow the volatiles to separate from the sample. Purge time was 11 min at 30°C in a Trap10 trap (O-I-Analytical, College Station, TX). After purging, the volatiles were desorbed 4 min at 190°C. The subsequent run on the GC/MS (Agilent Technologies 6890N Network GC system) used a 30 m x 0.25 mm ID x 0.25 μm
film thickness column. The volatile compounds were held at 40°C for four min followed by an 8°C/min ramp to 250°C and held for 10 min. Compound masses were then determined with a mass spectrometer (Agilent Technologies 5973 inert Mass Selective Detector) and identified by the database (NIST/EPA/NIH Mass Spectral Library, Version 2.0a).

3. Results and discussion

3.1 SPME comparison with Purge and Trap

Hexanal, pentane, and propanal were run as standards in the SPME method so concentration could be determined for those compounds. Other samples were just identified by the mass spectrometer as present or absent.

The SPME results for *M triceps brachii* were very similar to the results from the purge and trap-MS results (Table 1). Three compounds having different concentrations between the normal and liver-like samples with purge and trap (heptanal, 2-methyl-3-octanone, and 2-octenal) were not identified in any of the samples with SPME. Two alkanes, pentane and hexane, were identified with SPME and not with purge and trap. Propanal was shown to have approximately 3.3 times higher concentration in the liver-like samples with the SPME method. All three of those compounds have lower molecular weights than any of the compounds picked up with the purge and trap method.

Additionally, limonene was not identified with purge and trap-MS, but other studies, such as Yancey et al. (2006), have also identified limonene in liver-like samples. However, β-pinene is a major breakdown product of limonene so it is thought the purge and trap-MS is picking up β-pinene instead of limonene. While not quantified, octanol in the normal sample was barely at the detection level in the SPME assay, while the liver-like sample
clearly showed that octanol was present. The same trend was seen in with the purge and trap samples. The hypothesis exists that methylbutanal is one of the early-eluting, unknown compounds in the liver-like samples since it was a similar peak to the one found in raw liver samples with the same retention time (p. 189).

Comparisons of results with the same samples between SPME and the purge and trap method seem to yield similar results. The purge and trap method seems to be able to detect some additional compounds, while SPME does a much better job identifying the lower molecular weight compounds. Further work to separate the lower molecular weight compounds in the purge and trap method is needed especially to investigate the effect of propanal has on the liver-like flavor. Lowering the initial oven temperature or running with cryogenic conditions on the purge and trap-MS might solve this issue.

### 3.2 Verification of compounds found in the M. rectus femoris with Purge and Trap MS

The verification of results by running six additional *M. rectus femoris* samples from the innovative selection procedure (Jenschke et al., 2006) yielded further information including possible identification of two previous unknowns of difluorochloromethane and trichloromethane. The *M. rectus femoris* from the innovative selection process all contained an additional volatile compound, toluene, with a retention time of 5.25 min. Interestingly, with the initial samples that were used to create the volatile compound list of differences between liver-like and normal muscles, 1-Hexanol, 2-ethyl- was identified whereas this run always identified the compound at the retention time of 12.10 min to be limonene. The presence of limonene has been cited in other references as well (Yancey et al., 2006). While the samples from this trial were originally designed to show comparisons between liver-like and normal samples, the
samples were not found to be liver-like in the sensory trial (Jenschke et al., 2006), and the purge and trap-MS results support that finding. Differences in the peak concentration of compounds between the Choice and Select do not seem to be visually different. The remainder of the compounds were very similar to the results from the original trial normal samples. Based on these results, the purge and trap-MS method gives repeatable results when raw muscle samples are utilized. Further testing of the method when liver-like samples are identified by taste panel will be needed to verify the differences seen between liver-like and normal samples. Due to the minimal compound differences (Hodgen et al., 2006) in the M. rectus femoris between the normal and liver-like samples, another muscle should be used to validate the results.

4. Conclusion
The SPME method validated the purge and trap-MS method. Additional work to identify lower molecular compounds would enhance the ability of the purge and trap to pick up differences in liver-like and normal samples. Running more muscle samples with the purge and trap-MS methodology verified the normal muscle results seen with the initial trials run. Additional testing with more liver-like samples is needed to establish that differences seen in liver-like and normal samples were correct.
Acknowledgements

1 This paper is a contribution of the University of Nebraska Agricultural Research Division.

2 This project was funded in part by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen’s Beef Board and state beef councils by the National Cattlemen’s Beef Association.

3 A special thanks is extended to Daniel Snow and David Cassada in the UNL Water Resources Department for their assistance with the mass spectrometry work.
References


Table 1. Volatiles identified with SPME compared with ones identified from Purge and Trap Method for the *M. triceps brachii*.

<table>
<thead>
<tr>
<th>Compounds From P&amp;T&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SPME Normal Sample</th>
<th>SPME Liver-like Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentanal&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Heptanol&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexanal&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>2X</td>
</tr>
<tr>
<td>2-Heptanone&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Heptanal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Benzaldehyde&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>β-Pinene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>1-Octen-3-ol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2-Methyl-3-octanone&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Pentylfuran&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Octanol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Near detection level</td>
<td>X</td>
</tr>
<tr>
<td>α-Pinene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2-Octenal&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Octenal&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Nonanal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Additional SPME Compounds**<sup>b</sup>

| Pentane<sup>b</sup>          | X                  |
| Hexane<sup>b</sup>           | X                  |
| Propanal<sup>b</sup>         | X                  | 3.3X                   |
| Methylbutanal<sup>b</sup>    | X                  | X                      |
| Butenal<sup>b</sup>          | X                  | X                      |
| Limonene<sup>b</sup>         |                    | X                      |

<sup>a</sup> Compounds were identified as having different concentrations between the normal and liver-like samples in the purge and trap method.

<sup>b</sup> Additional compounds identified with the SPME method. Some of these compounds were identified with purge and trap but were not visually different in concentration.

<sup>c</sup> Hexanal, pentane, and propanal were run as standards in the SPME method so concentration could be determined for those compounds. The number in front of the ‘X’ indicates how much higher the concentration was in that sample. Other SPME isolated compounds were just identified by the mass spectrometer as present or absent.

<sup>d</sup> A very small peak was present in the SPME Normal sample with the same retention time as octanol but was not at the threshold level to be identified by mass spectrometry.
Recommendations for Future Work

Cooking Rate and Holding Time

Several topics of interest developed from the initial study that have not been explored yet.

1) With a larger sample size and having enough sample of all the muscles (i.e. *M. teres major*), would the interaction of cooking rate and holding time have been significant? More samples probably would have lead to this conclusion, but the limited degrees of freedom did not allow for this comparison to be adequately tested.

2) Extending the holding time for longer than 1 h needs to be investigated. Belk et al. (1993) found there was an increase in off-flavors perceived as holding time increased past 1 h for foodservice roasts. Does this trend hold true with all beef cuts or are there particular cuts that are better suited for maintaining quality with longer holding times? Both traditional beef items and the value cuts need to be explored.

3) Addressing issues of enhancement. The industry is moving toward more enhanced and marinated products for several reasons, including ease of preparation and consistency of the final product. If foodservice follows the same trend, the effects of cooking rate and holding time on the eating quality of these enhanced or marinated products needs to be investigated. This approach of enhancement may also play a role in reducing the incidence of off-flavors and complaints from customers when served steak-like products without additional sauces to mask the undesirable off-flavors.
4) A major interest that both we and foodservice providers have expressed is the plumping of some of the beef value cuts when cooking. Plumping is an issue because, in order to gain adequate internal temperature, the steak has a charred outer appearance and charred taste, especially when cooking on high heat. Determining the cause of the plumping phenomena and finding a solution will be beneficial in terms of meat quality and food safety.

*Flavor Research*

The biggest hurdle for flavor research in beef, especially in regards to the liver-like off-flavor, is finding enough samples. The approach of screening samples in the plant did not yield favorable results due to small amounts of variation in the population. There are multiple reasons for this including no bad samples, air quality at the plant, many other undesirable flavors (metallic, sour, organic) in the meat, the muscle chosen as the indicator muscle, etc. Based on the mass spectrometry results, the *M. rectus femoris* was a poor choice as an indicator muscle for these innovative selection trials as it had the fewest differences in compounds between the normal and liver-like beef. Moreover, the concentration of all the compounds in the *M. rectus femoris* was in higher concentration, in most cases, than other value cut muscles. For the innovative selection panel to work, the screening would probably have to be run on each specific muscle with an understanding of the particular aromas of the compounds that will cause the off-flavor in that muscle. This unfortunately means many samples of the off-flavored meat would need to be available for training of the panel that would conduct the in-plant screening.
Further Comments:

1) The expertise of an organic chemist and/or a flavor chemist is needed. No further research can be conducted without gaining some insight from these types of experts. Understanding of chemical compound interactions would shorten the time of discovery of the cause of the problem. Additionally, their background knowledge of possible origins of these compounds would be useful.

2) More work is needed to establish further protocols for the mass spectrometry work done at UNL on meat. Should the samples be flushed with gas prior to running a sample to eliminate some of the background noise or possible interactions if the samples are not run immediately? What is the best temperature to run meat samples? This needs to be addressed for both raw and cooked samples. In the preceding studies, 40°C and 80°C were used. Is some temperature in between better? Based on the verification and validation study, efforts to identify lower molecular weight compounds are necessary. Starting with a lower initial oven temperature might help. Using the cryogenic capabilities of the purge and trap system, the issue might also be resolved.

3) Investigate the volatile compounds from cooked samples. Because the samples were not cooked prior to the mass spectrometry run, no Maillard or Strecker degradation products from the heating process were present. Other meat studies have shown there to be differences in various pyrazines, thiols, and furans when the samples were handled differently or were from different
sources. Further information about the cause of the liver-like flavor might be obtained.

4) With the compounds known presently that appear to contribute to a muscle being liver-like, it might be possible to develop a gas chromatography protocol that would save the expense of running each sample through the mass spectrometer. This protocol could also be used to complete the work done in study 2 of this dissertation which was abandoned once the purge and trap system was installed.

5) Validation of all the muscles with SPME might be interesting.

6) With the interest in profiling all characteristics of beef and pork muscles, it might be useful to obtain raw and cooked aroma profiles from all muscles of interests. Working with a knowledgeable flavor chemist with culinary interests could prove very valuable in increasing the utilization of various muscles or enhancing the natural ‘potential’ of each particular muscle.

7) There is a basic start to understanding the compounds that differentiate the liver-like and normal samples. As the research continues, the use of an objective screening method, such as an electronic nose, will become necessary.

8) Most of the compounds of interest found in the mass spectrometry study were related to oxidation. However, Yancey et al. (2006) found no relationship with lipid oxidation. Several groups working with flavor in meat have come to the conclusion that the TBARS procedure is not the best method to determine lipid oxidation in samples because this procedure just takes into
account the pathway to produce malonaldehyde. This does not allow for oxidation products of longer polyunsaturated fatty acids that also have the potential to create oxidation byproducts. No ideal solution to this issue has been addressed although numerous authors have also recognized this problem. At this point, the best method (once calibrated and verified) would be GC-MS. Looking at the compounds present and understanding where each was derived from (linoleic acid, arachidonic acid, etc.) would be important in developing this method. A suggestion from early in this research was to change the atmosphere in meat packages to create different oxidative conditions to promote different pathways of oxidation and see flavor differences. This might also create enough off-flavored samples that further GC-MS work could be conducted.

9) Because the innovative screening method was not completely successful, the use of trained panelists will continue. Technology has introduced an apparatus that is fitted into a panelist’s nose, and sends the compounds obtained to a mass spectrometer. Even though this technology is expensive, it would be very useful with this type of research.

   a. The gas chromatograph/mass spectrometry-olfactory system (GC/MS-O) breaks the individual compounds down according to molecular weight, ionic charge, etc. It fails to take into account the mastication process. The interaction of salvia when chewing produces additional compounds that we cannot reproduce with the standard procedures. Since the liver-like flavor has always been believed to be an aromatic
and is usually strongest after chewing has begun, the additional variable of mastication should be included when trying to determine the compounds of interest.

b. This instrument also has the benefit of addressing two issues at once. One gets both the mass spectrometric results as well as gaining valuable sensory information while only using one small piece of meat.

Investigating the Serumy/Bloody Effect

Initially, the comparative study between liver and liver-like samples was also to look at beef blood and serum to investigate to see if improper exsanguination (too much blood left in the muscle) or serum might contribute to the off-flavor. This portion was originally designed by obtaining blood during the exsanguination of a beef animal. Half the blood had anticoagulant added and the other half did not. For each treatment (with or without coagulant), half was retained as whole blood and half was centrifuged to obtain serum and the solids of whole blood. Blood, serum, and solids were placed in vials and frozen until tests could be run.

The results revealed the blood and serum had too many free radicals/free fatty acids. During the purging phase so many bubbles were created that the vials could not maintain the pressure and the liquid and gases escaped. Perhaps with anti-foam this problem could have been alleviated. However, this being a sideline project of mine, I did not pursue it as the Water Resources Lab ran the samples once for free, but would not do so again. Additionally, time became a factor so this project got indefinitely postponed but might be of interest to explore later.
Determining Origins of Compounds

Because of the lack of liver-like samples available for testing, this issue becomes even more important. With the little data we have, is it possible to determine the origin of the compounds? To investigate possible origins, several key assumptions have to be believed true. These assumptions stem from our belief that if a carcass had a muscle that was off-flavored, many of the muscles in that carcass would be off-flavored. This was shown in the cooking rate and holding time study and followed up by Meisinger et al. (2006). What caused those three carcasses to be different from the others: genetics, pre-harvest environment, or post-harvest handling environment? Several studies, including the cooking rate and holding time study, have found minor effects due to post-harvest handling practices. Because genetics is an uncontrollable variable at this point, we chose to believe that something in the environment is contributing to the development of these undesirable off-flavors. This is further strengthened by the fact that numerous individuals from other countries, which do not feed corn-based diets, have said their meat has a liver-like characteristic. Thus, they do not find unacceptable what typical consumers in the USA find unacceptable. All of this circumstantial evidence points to a feedstuff, feed additive, or pharmaceutical being a culprit in producing the liver-like off-flavor.

Based on the compounds found, background searches into possible origins has not been easy due to the biohydrogenation process in the rumen. Assuming that only minor changes have taken place to the compounds in the rumen, consumption of shrubs such as creosote, tarbursh, and cedar trees might lead to the perception of liver-like off-flavor in the meat. There are problems with this line of thinking,
however. The most obvious is that most ruminants do not eat those types of plants unless no other food is available. Additionally, most cattle in the US are finished on a grain-based diet for the last 90 plus days of their lives. Would enough of the compound already be in the muscle to maintain a perceived off-flavor after that much turnover time? Perhaps other forages are unknown sources of the off-flavor in beef.

*Distillers Grains*

An additional hypothesis has been investigated by this laboratory as well: the increased use of distillers grains in the finishing diet increases the likelihood and/or intensity of off-flavor. The initial trial using the *M. rectus femoris* yielded no correlation between the flavor and the level of distillers grain feed. However, there are still several issues to consider before ruling out distillers grains as a suspect.

1) Possible reasons why distillers grains may be an origin of the compounds:

a. As stated in the mass spectrometry paper, many of the compounds seen were from oxidation of oleic and linoleic fatty acids. Feeding distillers grains to lambs will increase the unsaturated fatty acid concentration in the muscle (Vipond, Lewis, Horgan, & Noble, 1995). This theory is currently being investigated by this laboratory in beef muscle. How much of an increase is needed for the liver-like flavors to occur? Does it have more to do with aging and changes in shelf-life?

b. Because of the ethanol and co-product process, high levels of sulfur are used. Certain times of the year or specific ethanol manufacturing plants have higher levels of sulfur in the co-
products than is recommended for cattle, especially in Nebraska, where sulfur levels in animal drinking water are uncommonly high, as well. Sulfur issues with animals usually affect the liver as well as causing the animal to go off feed for extended periods of time or causing polioencephalomalacia (PEM, polio, “brainers”) reported in some feedlots. The preceding papers found no sulfur compounds causing differences in liver-like and normal samples. However, as stated earlier, the sulfur compound and their differences might show up if the samples were cooked to allow development of Maillard reaction products. Additionally, octanal and a sulfur compound in meat have the same mass and would be identified as octanal by the database. However, many sulfur compounds have low molecular weights and would come out much earlier in a GC-MS system than octanal so they should be identifiable if they were present. Therefore, the sulfur compound in meat that is found at the same retention time as octanal would not be vital to determine if sulfur was a cause in the off-flavor.

c. The purge and trap system is good for collecting aromatics, and the assumption we have been working with, as well as many others, is the liver-like off-flavor is aromatic. If the sulfur compound is not aromatic, then the purge and trap system would not be a suitable method for trying to pick up the differences.
d. Most of the work at UNL in respect to distillers grains has been with wet distillers grain plus solubles. It is possible that another co-product is the source of off-flavors. Corn gluten has been widely distributed to ranchers in the form of aid due to the drought. One would assume dried distillers grain would be more of an issue than wet distillers grain in producing off-flavors since the dried has moisture removed and the volatile compounds would be more concentrated (unless, when driving off the moisture, the water soluble aromatic is also driven off).

2) Possible reasons why distillers grains may not be the origin of the compounds:
   a. The liver-like off-flavor has been mentioned in past literature prior to the increased feeding of distillers grain as a finishing diet.
   b. Jenschke et al. (2006) found no correlation with distillers grains when fed rations of 0-50% of wet distillers grains + solubles. If distillers grains was a possible origin, a trend should have been seen in that study.
   c. While an increase in unsaturated fatty acids is desirable for nutritional benefits, could the liver-like flavor just be due to that? Other species have seen increases in unsaturated fatty acids without having reports of liver-like flavors. There would likely have to be some interaction with other compounds in the meat as
fatty acid composition in muscle is very hard to manipulate in a ruminant animal.

d. Many people report octanal as a compound of oxidation so based on results in this study, the compounds identified were too similar to other studies that looked at sulfur compounds for us to assume that sulfur was actually what was different in the samples not octanal, especially since octanal was not different in all the liver-like muscles compared to the normal muscles.
APPENDIX
APPENDIX 1
Example of Taste Panel Ballot

FLAVOR Taste Panel
DAY 1

Date: August 1, 2004

Panelist #: _______________

Please evaluate the flavor of the sample by using the rating scale (1-8) for off-flavor intensity and then identifying the flavor notes associated with the sample.

Rating Scales:

<table>
<thead>
<tr>
<th>TENDERNESS</th>
<th>CONNECTIVE TISSUE</th>
<th>JUICINESS</th>
<th>OFF-FLAVOR INTENSITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Extremely Tender</td>
<td>8 No Connective Tissue</td>
<td>8 Extremely Juicy</td>
<td>8 No Off-Flavor</td>
</tr>
<tr>
<td>7 Very Tender</td>
<td>7 Trace Amount</td>
<td>7 Very Juicy</td>
<td>7 Trace Off-Flavor</td>
</tr>
<tr>
<td>6 Moderately Tender</td>
<td>6 Slight Amount</td>
<td>6 Moderately Juicy</td>
<td>6 Slight Off-Flavor</td>
</tr>
<tr>
<td>5 Slightly Tender</td>
<td>5 Small Amount</td>
<td>5 Slightly Juicy</td>
<td>5 Small Off-Flavor</td>
</tr>
<tr>
<td>4 Slightly Tough</td>
<td>4 Modest Amount</td>
<td>4 Slightly Dry</td>
<td>4 Modest Off-Flavor</td>
</tr>
<tr>
<td>3 Moderately Tough</td>
<td>3 Moderate Amount</td>
<td>3 Moderately Dry</td>
<td>3 Moderately Off-Flavor</td>
</tr>
<tr>
<td>2 Very Tough</td>
<td>2 Slightly Abundant</td>
<td>2 Very Dry</td>
<td>2 Very Off-Flavor</td>
</tr>
<tr>
<td>1 Extremely Tough</td>
<td>1 Abundant Amount</td>
<td>1 Extremely Dry</td>
<td>1 Extreme Off-Flavor</td>
</tr>
</tbody>
</table>

Sample ID | Tenderness | C.T. | Juiciness | O.F. Intensity | Flavor/O.F. Description | Comments |
----------|------------|------|-----------|----------------|-------------------------|----------|
348       |            |      |           |                |                         |          |
876       |            |      |           |                |                         |          |
549       |            |      |           |                |                         |          |
428       |            |      |           |                |                         |          |
906       |            |      |           |                |                         |          |
675       |            |      |           |                |                         |          |
148       |            |      |           |                |                         |          |
160       |            |      |           |                |                         |          |

1 = Liver-like     5 = Oxidized/ Warmed Over
2 = Metallic       6 = Rancid
3 = Sour/Acidic    7 = Other
4 = Charred/Bitter 8 = None
APPENDIX 2

COMPOUND INFORMATION

Compiled from:

Flavornet (www.flavornet.org)
Leon Lab (http://leonlab.bio.uci.edu/odorants.cfm)

Accessed 9-21/22-2006

APPENDIX 2.1

BENZALDEHYDE

Also known as:

benzoic acid, artificial essential oil of almond

FACTS:

C\textsubscript{7}H\textsubscript{6}O

106.12 g/mol

CAS 100-52-7

Narcotic in high concentrations and may cause dermatitis

CHARACTERISTIC FLAVOR/AROMAS:

Odor of Volatile Oil of Almond, Burning Aromatic Taste, Bitter Almond

COMMON USES

Manufacture of dyes, perfumery, cinnamic, and mandelic acids, solvents, flavors
APPENDIX 2.2

BENZENE

Also known as:
benzol; cyclohexatriene

FACTS:

$C_6H_6$

78.11 g/mol

CAS 71-43-2

CHARACTERISTIC FLAVOR/AROMAS:

Pleasant, Distinct

COMMON USES

Industrial Uses
APPENDIX 2.3

sec-BUTANAMINE

Also known as:
2-butanamine; 2-butylamine; 2-aminobutane; frucote; deccotane; tutane

FACTS:
C₄H₁₁N
73.14 g/mol
CAS 513-49-5

CHARACTERISTIC FLAVOR/AROMAS:
Seafood, Green, Onion

COMMON USES
Fungistat
APPENDIX 2.4

BUTENAL

Also known as:
2-Butenal, Crotonaldehyde, β-methyl acrolein, but-2-enal, methylpropenal,
propylene aldehyde

FACTS:

\[ \text{C}_4\text{H}_6\text{O} \]
70.09 g/mol
CAS 123-73-9

CHARACTERISTIC FLAVOR/AROMAS:
Malty, Green, Roast

COMMON USES
Solvents
APPENDIX 2.5

*n-CAPROIC ACID, VINYL ESTER

\[
\text{C}_6\text{H}_{12}\text{O}_2
\]

Also known as:
hexanoic acid

FACTS:

C\text{6}H\text{12}O\text{2}

116.16

CAS 142-62-1

CHARACTERISTIC FLAVOR/AROMAS:

Goaty Odor
APPENDIX 2.6

3-CARENE

Also known as:

3,7,7-trimethylbicyclo[4.1.0]hept-3-ene; Δ-carene; 4,7,7-trimethyl-3-norcarene;
isodiprene

FACTS:

C_{10}H_{16}

136.23 g/mol

CAS

constituent of turpentine

CHARACTERISTIC FLAVOR/AROMAS:

Sweet & Pungent Odor, but more agreeable in odor than turpentine, Orange Peel,

Lemon, Resin
APPENDIX 2.7

CYCLOBUTANOL

Also known as:

hydroxycyclobutane

FACTS:

C₄H₈O

72.11 g/mol

CAS 2919-23-5

CHARACTERISTIC FLAVOR/AROMAS:

Roasted
APPENDIX 2.8

CYCLOHEXANONE, 2,2,6-TRIMETHYL

Also known as:
Decadienal, Heptenyl acrolein

FACTS:

C₉H₁₆O

140.23 g/mol

CAS 2408-37-9

CHARACTERISTIC FLAVOR/AROMAS:

Mint, Acetone
APPENDIX 2.9

2,4-DECADIENAL

Also known as:
Decadienal, Heptenyl acrolein

FACTS:

C_{10}H_{16}O

152.23 g/mol

CAS 25152-84-5

CHARACTERISTIC FLAVOR/AROMAS:

Seaweed

Deep fat flavor, characteristic chicken aroma at 10ppm; Citrus/orange/grapefruit character at lower dilution.

10 ppm use to impart a deep fat flavor in beef, lamb, chicken, potato chips and french fries

1 ppm use to fortify orange-grapefruit flavors
APPENDIX 2.10

DECANAL

Also known as:
Capric aldehyde

FACTS:
$C_{10}H_{20}O$
156.3 g/mol
CAS 112-31-2

CHARACTERISTIC FLAVOR/AROMAS:
Powerful, Waxy, Aldehydic, Orange Character, Citrus Peel

COMMON USES
Fragrances
APPENDIX 2.11

2-DECENAL

Also known as:
2-Decen-1-al; Decenaldehyde; Decylenic aldehyde; 3-Heptyl acrolein; Dec-2-enal

FACTS:

\[ \text{C}_{10}\text{H}_{18}\text{O} \]

154.25 g/mol

CAS 3913-81-3

CHARACTERISTIC FLAVOR/AROMAS:

Tallow, Orange
APPENDIX 2.12

1,3-BIS(1,1-DIMETHYLETHYL)BENZENE

Also known as:

1,3-bis(1,1-dimethylethyl)benzene; 1,3-\textit{tert}-dibutylbenzene; \textit{m}-Di-\textit{tert}-butylbenzene;

benzene, 1,3-bis(1,1-dimethylethyl)-; 1,3-\textit{Di-tert}-butylbenzene; \textit{tert}-butylbenzene;

FACTS:

\[
\text{C}_{14}\text{H}_{22}
\]

190.32 g/mol

CAS 1014-60-4

CHARACTERISTIC FLAVOR/AROMAS:

Cooked Beef
APPENDIX 2.13

2,3-DIMETHYLOXIRANE

Also known as:

*meso*-2,3-epoxy-butane; 2,3-dimethyloxirane; *cis*-2,3-Dimethyloxirane; (2S,3R)-
2,3-Dimethyl-oxirane; Oxirane, 2,3-dimethyl- (2R,3S)-rel-; *cis*-2,3- Epoxybutane;

*cis*-β-Butylene Oxide

FACTS:

\[ \text{C}_4\text{H}_8\text{O} \]

72.11 g/mol

CAS 1758-33-4
APPENDIX 2.14

1,2-ETHANEDIAMINE, N, N’-DIMETHYL

Also known as:

1,2-diamino-ethaan; 1,2-diamino-ethano; 1,2-diaminoaethan; 1,2-diaminoethane;
1,2-ethanediamine; aethaldiamin; ethylene diamine; ethylenediamine

FACTS:

C₂H₈N₂
60.10 g/mol
CAS 107-15-3

CHARACTERISTIC FLAVOR/AROMAS:

Ammonia

COMMON USES

Solvents
APPENDIX 2.15

5-ETHYLCYCLOPENT-1-ENECARBOXALDEHYDE

FACTS:

CAS 31906-04-4

Common Fragrance allergen

CHARACTERISTIC FLAVOR/AROMAS:

Fragrant; Perfume
APPENDIX 2.16

FURAN, 2-PENTYL

Also known as:

pentylfuran, 2-amylfuran

FACTS:

C_9H_{14}O

138.21 g/mol

CAS 3777-69-3

Low detection threshold

CHARACTERISTIC FLAVOR/AROMAS:

Green Bean, Butter
APPENDIX 2.17

2,4-HEPTADIENAL

Also known as:

(E,E)-2,4-heptadienal; (2E,4E)-hepta-2,4-dienal

FACTS:

C\textsubscript{7}H\textsubscript{10}O

110.1 g/mol

CAS 4313-03-5

CHARACTERISTIC FLAVOR/AROMAS:

Nut, Fat
APPENDIX 2.18

HEPTANAL

Also known as:
heptaldehyde, aldehyde C-7, heptyl-aldehyde, oenanthal, enanthal, oenanthol,
oenanthaldehyde, enanthaldehyde

FACTS:

$C_7H_{14}O$

114.18 g/mol

CAS 111-71-7

CHARACTERISTIC FLAVOR/AROMAS:

Oily-Fatty, Rancid, Unpleasant

(In Liquid) Penetrating Fruity Odor, Fermented Fruit
APPENDIX 2.19

2-HEPTANAMINE, 5-METHYL

Also known as:
Tuaminoheptane; 2-heptanamine; 1-methylhexylamine; 2-aminoheptane;
heptamine; heptin; heptedrine; tuamine; octodrine

FACTS:

C₈H₁₉N

129.24 g/mol

CAS 53907-81-6
APPENDIX 2.20

1-HEPTANOL

Also known as:

n-heptyl alcohol, enanthic alcohol, 1-hydroxyheptane, n-heptanol

FACTS:

C\textsubscript{7}H\textsubscript{16}O

116.20 g/mol

CAS 111-70-6

CHARACTERISTIC FLAVOR/AROMAS:

Fragrant, Woody, Oily, Green, Fatty, Winey, Sap, Herb
APPENDIX 2.21

2-HEPTANONE

Also known as:

methyl amyl ketone

FACTS:

C\textsubscript{7}H\textsubscript{14}O

114.18 g/mol

CAS

CHARACTERISTIC FLAVOR/AROMAS:

Peppery odor in cheese, fruity, spicy, cinnamon (found in cloves and cinnamon bark oil)

(In liquid) penetrating fruity
APPENDIX 2.22

2-HEPTANONE, 6-METHYL

Also known as:
6-methyl-heptan-2-one; 6-Methyl-2-heptanone; 2-Methyl-6-heptanone; Methyl isohexyl ketone; 6-methylheptan-2-one; isohexyl methyl ketone; 6-Methyl-2-heptanon

FACTS:

$C_8H_{16}O$

128.21 g/mol

CAS 928-68-7

Component in deer scent

CHARACTERISTIC FLAVOR/AROMAS:

Cloves, Menthol, Eugenol
APPENDIX 2.23

2-HEPTENAL

Also known as:

heptenal

FACTS:

C$_7$H$_{12}$O

112.17 g/mol

CAS 18829-55-5

CHARACTERISTIC FLAVOR/AROMAS:

Soapy, Fatty, Almond, Fishy, Unpleasant
APPENDIX 2.24

1,3-HEXADIENE, 3-ETHYL-2-METHYL

\[
\begin{align*}
\text{H}_2\text{C} & = \text{C} \sim \text{C} = \text{C} & \text{C}_2\text{H}_5 \\
\text{H} & \quad \text{H} & \\
\end{align*}
\]

FACTS:

\[\text{C}_2\text{H}_5\text{CH} = \text{CHCH} = \text{CH}_2\]

CAS 61142-36-7
APPENDIX 2.25

HEXANAL

Also known as:
hexyl aldehyde, hexanaldehyde, caproaldehyde, caproic aldehydes, hexaldehyde

FACTS:

$\text{C}_6\text{H}_{12}\text{O}$

100.18 g/mol

CAS: 66-25-1

CHARACTERISTIC FLAVOR/AROMAS:
Fatty-Green, Grassy, Strong Green, Tallow, Fat
(When Dilute) Unripe Fruit, Soft Fruity

COMMON USES
flavoring additive in food, used in the creation of plasticizers, rubber, dyes, plastic
resins and insecticides.
APPENDIX 2.26

HEXANE

Also known as:

$n$-Hexane

FACTS:

$C_6H_{14}$

86.17 g/mol

CAS 110-54-3

CHARACTERISTIC FLAVOR/AROMAS:

Faint, peculiar odor

COMMON USES

Determining Refractive index of minerals,

Filling for thermometers
APPENDIX 2.27

HEXANOL

Also known as:

\( n\)-hexyl alcohol, 1-hexanol, amylcarbinol, pentylcarbinol, 1-hydroxyhexane

FACTS:

\[ \text{C}_6\text{H}_{14}\text{O} \]

102.17 g/mol

CAS 111-27-3

CHARACTERISTIC FLAVOR/AROMAS:

Woody, Cut Grass, Chemical-Winey, Fatty, Fruity, Weak Metallic

COMMON USES

Antiseptics, hypnotics
APPENDIX 2.28

1-HEXANOL, 2-ETHYL

Also known as:
2-ethylhexanol, 2-ethylhexan-1-ol, 2-ethylhexyl alcohol,

FACTS:
\[ \text{C}_8\text{H}_{18}\text{O} \]
130.23 g/mol
CAS 104-76-7

CHARACTERISTIC FLAVOR/AROMAS:
Resin, Flower, Green

COMMON USES
Solvent
APPENDIX 2.29

2-HEXEN-1-OL

Also known as:
Hex-2-Enol; gamma-Propyl Allyl Alcohol; Leaf Alcohol; trans-2-hexen-1-ol; (E)-3-propyl allyl alcohol; trans-hex-2-en-1-ol; (E)-2-hexenol

FACTS:

\[ \text{C}_6\text{H}_{12}\text{O} \]

100.16 g/mol

CAS 928-95-0

Naturally occurs in apple, grape, kiwi fruit, mango, orange juice, pineapple,
raspberry, rice, strawberry, tea green, tomato

CHARACTERISTIC FLAVOR/AROMAS:

Green (odor strength=High, recommend smelling in a 10.00 % solution or less)

sharp green leafy fruity unripe banana

At 2.00 - 9.00 ppm. Green leafy, fresh, fatty, grassy with fruity and juicy nuances
APPENDIX 2.30

HYDROXYMANDELIC ACID

Also known as:
vanilmandelic acid (VMA); α, 4-dihydroxy-3-methoxybenzeneacetic acid; 3-methoxy-4-hydroxymandelic acid; 4-hydroxy-3-methoxymandelic acid

Misnamed: vanillinemandelic acid and vanillylmandelic acid

FACTS:

C_{9}H_{10}O_{5}

198.17 g/mol

CAS 17119-15-2

COMMON USES

Catecholamine metabolite
APPENDIX 2.31

LIMONENE

Also known as:
1-Methyl-4- (1-methylethenyl) cyclohexene; p-mentha-1-8-diene; cinene;
cajeputene; kautschin

FACTS:

C_{10}H_{16}

136.23 g/mol

CAS 5989-54-8

CHARACTERISTIC FLAVOR/AROMAS:
Pleasant Lemon-like, Turpentine, Citrus, Fruity, Fresh, Light

COMMON USES

Solvent, Perfume, Flavorings
APPENDIX 2.32

MALONIC ACID, BIS(2-TRIMETHYLSILYETHYL ESTER)
APPENDIX 2.33

3-METHYLBUTANAL

Also known as:
Isovaleraldehyde; isovaleral; isovaleric aldehydes; methylbutanal

FACTS:

$C_5H_{10}O$

86.13 g/mol

CAS 590-86-3

Occurs in orange, lemon, peppermint, eucalyptus and other oils

CHARACTERISTIC FLAVOR/AROMAS:

Pungent Apple-Like Odor, Malt

COMMON USES

Artificial flavors and perfume
APPENDIX 2.34

METHYL SALICYLATE

\[
\text{\begin{center}
\includegraphics[width=0.2\textwidth]{methyl_salicylate.png}
\end{center}}
\]

Also known as:

2-Hydroxybenzoic acid methyl ester

FACTS:

\[ \text{C}_8\text{H}_8\text{O}_3 \]

152.14 g/mol

CAS 119-36-8

Produced in small amount sin numerous plants as a pheromone and exopheromone

CHARACTERISTIC FLAVOR/AROMAS:

Cooling Sensation; Wintergreen; Gaultheria

COMMON USES

Deep heating linaments, Flavorings in small amounts, Odor masker for some pesticides
APPENDIX 2.35

2,4-NONADIENAL

Also known as:

(2E,4E)-nona-2,4-dienal; trans,trans-2,4-Nonadienal

FACTS:

C₉H₁₄O

138.1 g/mol

CAS 5910-87-2

CHARACTERISTIC FLAVOR/AROMAS:

Fat, Wax, Green, Watermelon, Geranium, Pungent
APPENDIX 2.36

NONANAL

Also known as:

nonyl aldehyde, aldehydes C-9, n-nonyl aldehyde, nonoic aldehyde,
pelargonaldehyde, 1-nonyl aldehyde, pergonal

FACTS:

\[ \text{C}_9\text{H}_{18}\text{O} \]

142.1 g/mol

CAS 124-19-6

CHARACTERISTIC FLAVOR/AROMAS:

Floral, Citrus, Fatty, Grassy, Waxy, Green
APPENDIX 2.37

2-NONANONE

Also known as:

nonan-2-one; methyl heptyl ketone; heptyl methyl ketone; β-nonanone

FACTS:

\[ \text{C}_9\text{H}_{18}\text{O} \]

142.1 g/mol

CAS 821-55-6

Naturally occurs in beer, butter, carnation, cheese blue, cheese cheddar, coconut, potato chip, rice cooked, rue, plant, strawberry

CHARACTERISTIC FLAVOR/AROMAS:

Hot Milk, Soap, Green, Fruity, Floral
APPENDIX 2.38

2-NONENAL

\[
\text{\begin{array}{c}
\text{O} \\
\end{array}} \]

Also known as:

*trans*-2-nonenaldehyde

FACTS:

\[ \text{C}_9\text{H}_{16}\text{O} \]

140.23 g/mol

CAS 2463-53-8

Widely found in nature: beer, coffee, cucumbers, watermelon, palm oil, potatoes, carrots, etc

CHARACTERISTIC FLAVOR/AROMAS:

Cardboardy, Orris, Fat, Cucumber, Paper

COMMON USES

Insect repellent
APPENDIX 2.39

OCTADECANAL

Also known as:
Octadecanaldehyde

FACTS:

$\text{C}_{18}\text{H}_{36}\text{O}$

268.48 g/mol

CAS 638-66-4

CHARACTERISTIC FLAVOR/AROMAS:

Oil
APPENDIX 2.40

OCTANAL

Also known as:
caprylic aldehydes, caprylaldehyde, octaldehyde, octyl aldehyde

FACTS:

$C_8H_{16}O$

128.21 g/mol

CAS 124-13-0

CHARACTERISTIC FLAVOR/AROMAS:

Harsh, Fatty, Orange Peel, Soapy, Lemon, Green

(When Dilute) Sweet, Orange, Honey
APPENDIX 2.41

1-OCTANOL

Also known as:

octanol, octyl alcohol, -alcohol C-8, caprylic alcohol, capryl alcohol

FACTS:

C₈H₁₈O

130.22 g/mol

CAS 111-87-5

CHARACTERISTIC FLAVOR/AROMAS:

Penetrating Aromatic Odor, Fatty, Waxy, Citrus, Oily, Walnut, Moss, Chemical,

Metal, Burnt
APPENDIX 2.42

*3-OCTANONE, 2-METHYL

Also known as:
2-methyl-3-octanone, 2-methyloctan-3-one, n-amyl isopropyl ketone, isopropyl pentyl ketone

FACTS:

C$_9$H$_{18}$O

142.24 g/mol

CAS 923-28-4

CHARACTERISTIC FLAVOR/AROMAS:

Herb, Butter, Resin, Gasoline
APPENDIX 2.43

2-OCTENAL

Also known as:
Oct-2-Enal, 2-Octen-1-al; 2-Pentyl Acrolein; α-Amyl Acrolein

FACTS:

$\text{C}_8\text{H}_{14}\text{O}$

126.1 g/mol

CAS 2548-87-0

CHARACTERISTIC FLAVOR/AROMAS:

Green, Nut, Fat
APPENDIX 2.44

(Z)-3-OCTENE

\[
\text{C}_8\text{H}_{16} \quad \text{C=CH}_2 \quad \text{C}_2\text{H}_5
\]

Also known as:

cis-3-octene; cis-oct-3-ene; \( \gamma \)-cis-octene; 3-octene, (Z)-

FACTS:

\[
\text{C}_8\text{H}_{16}
\]

112.21 g/mol

CAS 14850-22-7

CHARACTERISTIC FLAVOR/AROMAS:

Fruity, Old Apples
APPENDIX 2.45

1-OCTEN-3-OL

Also known as:

vinyl pentyl carbinol, pentyl vinyl carbinol, ethyl vinyl carbinol, 1-penten-3-ol, n-amyl vinyl carbinol, n-pentyl vinyl carbinol, octenol

FACTS:

$C_8H_{16}O$

128.21 g/mol

CAS 3391-86-4

CHARACTERISTIC FLAVOR/AROMAS:

Mushrooms, Compound excreted by many insects
APPENDIX 2.46

2-OCTEN-1-OL

\[ \text{H}_2\text{O} \quad \text{CH} \quad \text{CH} \quad \text{CH} \quad \text{CH} \quad \text{CH} \quad \text{CH} \quad \text{CH} \]

Also known as:

trans-2-octen-1-ol, (E)-oct-2-en-1-ol, (E)-2-octenol

FACTS:

\[ \text{C}_8\text{H}_{16}\text{O} \]

128.22 g/mol

CAS 18409-17-1

CHARACTERISTIC FLAVOR/AROMAS:

Green Citrus
APPENDIX 2.47

3-OCTEN-2-ONE

Also known as:

Oct-3-en-2-one; Methyl Hexenyl Ketone

FACTS:

C₈H₁₄O

126.20 g/mol

CAS 1669-44-9

Naturally occurs in heated chicken, filbert roasted, fenugreek, potato baked, rice cooked

CHARACTERISTIC FLAVOR/AROMAS:

Nut, Crushed Bug, Earthy, Spicy, Herbal, Sweet, Mushroom, Hay, Blueberry

At 5.00 ppm. Creamy, earthy, oily with mushroom nuances
APPENDIX 2.48

OXIRANE, HEXADECYL

Also known as:
1,2-epoxyoctadecane, octadecene epoxide

FACTS:

C_{18}H_{36}O

268.478 g/mol

CAS 7390-81-0
APPENDIX 2.49

PENTANAL

\[
\begin{array}{c}
\text{O} \\
\text{H H H H H} \\
\text{H-C-C-C-C-C-H} \\
\text{H H H H H}
\end{array}
\]

Also known as:
pentyl aldehyde, valeraldehyde, valeral, valeric aldehyde

FACTS:

\[\text{C}_5\text{H}_{10}\text{O}\]

86.13 g/mol

CAS 110-62-3

CHARACTERISTIC FLAVOR/AROMAS:

Almond, Malt, Pungent, Acrid,

(When Dilute) Musty, Dry-fruity
APPENDIX 2.50

PENTANE

Also known as:

n-Pentane

FACTS:

C\textsubscript{5}H\textsubscript{12}

72.15 g/mol

CAS 109-66-0

CHARACTERISTIC FLAVOR/AROMAS:

Very Slight Warmed Over Flavor, Oxidized
APPENDIX 2.51

1-PENTANOL

\[ \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \]
\[ \text{HO-C-C-C-C-C-H} \]
\[ \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \]

Also known as:

Pentyl alcohol; n-amyl alcohol; n-butyl carbinol; pentan-1-ol; \( n \)-pentan-1-ol

FACTS:

\( \text{C}_5\text{H}_{12}\text{O} \)

88.15 g/mol

CAS 71-41-0

CHARACTERISTIC FLAVOR/AROMAS:

Mild Odor, Fusel Oil, Fruit, Balsamic

COMMON USES

Solvent
1-PENTANOL, 5-AMINO

Also known as:
5-amino-1-pentanol; 5-aminopentanol

FACTS:
C₅H₁₃NO
103.16 g/mol
CAS 2508-29-4

CHARACTERISTIC FLAVOR/AROMAS:
Mild
APPENDIX 2.53

α-PINENE

Also known as:
2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, 2-pinene, pinene

FACTS:

C_{10}H_{16}

136.23 g/mol

CAS 80-56-8

CHARACTERISTIC FLAVOR/AROMAS:

Piney, Fruity, Citrus

(Liquid) Characteristic Odor of Turpentine

COMMON USES

Manufacture of camphor, insecticides, solvents, plasticizers, perfume bases,
synthetic pine
APPENDIX 2.54

\[ \beta\text{-PINENE} \]

Also known as:
6,6-dimethyl-2-methylenebicyclo-[3.1.1]heptane, nopinene

FACTS:

\[ C_{10}H_{16} \]

136.23 g/mol

CAS 18172-67-3

The \(l\)-form occurs most commonly

Found in most essential oils with \(\alpha\)-pinene, but in smaller concentration

CHARACTERISTIC FLAVOR/AROMAS:

Pine, Citrus, Fruity, Resin, Turpentine
APPENDIX 2.55

PIPERAZINE

Also known as:

Hexahydropyrazine, Piperazidine
Diethylenediamine; 1, 4-Piperazine; 1,4-Diazacyclohexane; 1,4-Diethylenediamine;
1,4-Piperazine; Antepan; Antiren; Diethylenediamine; Diethyleneimine; Dispermine
; Entacyl; Eraverm; Eraverm; Hexahydro-1,4-diazine; Hexahydropyrazine;
Lumbrical; Piperazidine; Piperazine; piperazine (hexahydrate); Pipersol; Pyrazine
hexahydride; Pyrazine, hexahydro-; Tasnon; Upixon; Uvilon; Vermex; Vermizine;
Worm-A-Ton; Worm-away; Wurmirazin

FACTS:

C₄H₁₀N₂
86.14 g/mol
CAS 110-85-0

Piperazine is an organic compound that consists of a six-membered ring containing two
opposing nitrogen atoms. Piperazine exists as small alkaline deliquescent crystals with a
saline taste. The piperazines are a broad class of chemical compounds, many with
important pharmacological properties, which contain a core piperazine functional group. A
large number of piperazine compounds have anthelmintic action. Their mode of action is
generally by paralysing parasites, which allows the host body to easily remove or expel the
invading organism especially used as a wormer for roundworm. Piperazines are also used
in the manufacture of plastics, resins, pesticides, and other industrial materials. used as a
hardener for epoxy resins, an antihistamine, and an anthelmintic.

CHARACTERISTIC FLAVOR/AROMAS:

Salty Taste
APPENDIX 2.56

1,3-PROPANEDIAMINE, N-METHYL

Also known as:

N-Methyl-1,3-propanediamine; 3-(Methylamino)propylamine; N-Methyl-1,3-diaminopropane; 3-Methyl Amino Propyl Amine; 3-Methylaminopropylamine; 3-Methylamino-1-propylamine

FACTS:

C<sub>4</sub>H<sub>12</sub>N<sub>2</sub>

88.15 g/mol

CAS 6291-84-5
APPENDIX 2.57

PROPANOL

Also known as:
1-Propanol, propyl alcohol

FACTS:

\[ \text{C}_3\text{H}_8\text{O} \]

60.09 g/mol

CAS 71-23-8

CHARACTERISTIC FLAVOR/AROMAS:

Alcoholic and slightly stupefying odor

COMMON USES

As a solvent for resins and cellulose esters
APPENDIX 2.58

STYRENE

Also known as:

Ethenylbenzene, styrol, styrolene, cinnamene, phenylethylene, vinylbenzene

FACTS:

$C_8H_8$

104.14 g/mol

CAS 100-42-5

Low levels occur naturally in plants as well as a variety of foods such as fruits, vegetables, nuts, beverages, and meats.

CHARACTERISTIC FLAVOR/AROMAS:

Penetrating odor, Sweet smell
APPENDIX 2.59

TETRADECANE

Also known as:

\( n \)-tetradecane

FACTS:

\( \text{C}_{14}\text{H}_{30} \)

198.2 g/mol

CAS 629-59-4

CHARACTERISTIC FLAVOR/AROMAS:

Alkane
APPENDIX 2.60

TRIDECANE

Also known as:

$n$-tridecane

FACTS:

$C_{13}H_{28}$

184.22 g/mol

CAS 629-50-5

CHARACTERISTIC FLAVOR/AROMAS:

Alkane
APPENDIX 2.61

2-TRIDECENAL

Also known as:
Tridec-2-enal; 3-decyacroleinl tridecen-2-al-1

FACTS:

\[ \text{C}_{13}\text{H}_{24}\text{O} \]

196.33 g/mol

CAS 7774-82-5

CHARACTERISTIC FLAVOR/AROMAS:

Sweet, Strong, Spicy
APPENDIX 2.62

UNDEC-4-ENAL

COMMON USES

Animal pheromone