

DETERMINATION OF FAT PERCENTAGE USING THREE DIFFERENT
METHODS WITHIN MARBLING SCORES ON BEEF LONGISSIMUS MUSCLE

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DETERMINATION OF FAT PERCENTAGE USING CEM, ETHER AND FOLCH
FAT EXTRACTION METHODS WITHIN MARBLING SCORES ON BEEF
LONGISSIMUS MUSCLE

Danell Dow

Dr. Carol Lorenzen, Thesis Supervisor

ABSTRACT

Fat percentage determination in raw meat products has changed with technological advances. The development of rapid fat analysis methods has allowed the meat industry to implement these methods into commercial packing plants to aid in ensuring quality. The objective of this study was to determine fat percentage within marbling scores and compare three fat analysis procedures. Steaks (n = 119) were selected by USDA grading system using an E + V Vision Grading camera at a commercial beef plant during one day. Two samples per carcass were cut from the 13th rib, both sides, and transported to University of Missouri meat lab. The sample from the right side of the carcass was allotted to Warner-Bratzler shear force and the sample from the left side, which was graded by the camera, was allotted to fat extraction. Warner-Bratzler shear force samples were cut into 2.54 cm steaks and aged for 14 d. Steaks allotted to fat extraction were trimmed of all external fat and twice ground using 8 and 4 mm grinding plates. The finely ground beef was then split into its allotted fat extraction methods. The three methods used in fat extraction were 2:1 chloroform/methanol

(**Folch**), ether-extractable fat (**Ether**) and microwave drying and nuclear magnetic resonance (**CEM**). Warner-Bratzler shear force values were not different between marbling scores ($P > 0.05$). Regardless of fat extraction method, fat percentage increased as marbling score increased ($P < 0.05$). Regression equations for fat percentage using all extraction methods were linear. Prediction equation for CEM was fat percentage = $-3.46 + 0.016$ (marbling score), R^2 of 0.824 ($P < 0.0001$). Prediction equation for Ether was fat percentage = $-3.08 + 0.017$ (marbling score), R^2 of 0.859 ($P < 0.0001$). Folch prediction equation was fat percentage = $-3.42 + 0.019$ (marbling score), R^2 of 0.816 ($P < 0.0001$). When CEM, Folch and Ether methods were compared, CEM and Folch regression lines had different slopes ($P < 0.05$). The slope of the regression line for Ether was not different ($P > 0.05$) from CEM or Folch. Overall, tenderness was not affected by marbling score, but as expected, as marbling score increased fat percentage also increased regardless of fat extraction method.

Chapter 1

INTRODUCTION

Marbling is the number one factor in valuing beef carcasses both by industry and consumers. Marbling scores are used to determine USDA quality grade for carcasses and pricing at the wholesale level with higher quality grades commanding higher prices. Consumers perceive marbling as a basis for estimating eating quality and nutritional value of beef steaks. Eating quality is a combination of tenderness, juiciness and flavor. An increase in USDA quality grade has been shown to increase flavor, tenderness and overall palatability (Smith et al., 1987). Savell and Cross (1988) were able to create the *Window of Acceptability*, which resulted in an acceptable overall palatability in a fat range of 3 to 7.3% in beef *longissimus* steaks which translated to marbling scores ranging from Slight to Moderate.

In 1986, Savell et al. determined total fat percentage using ether-extractable fat within beef USDA marbling scores of *longissimus* muscle. Since 1986, cattle genetics and carcass traits have changed. According to the National Beef Quality Audits mean hot carcass weights (**HCW**), *longissimus* muscle area, marbling score, and USDA quality grade have increased (Lorenzen et al., 1993; Boleman et al., 1998; McKenna et al., 2000; Garcia et al., 2008). Also discounts are now given at 454 kg of HCW rather than 431 kg of HCW which has resulted in a trend toward heavier cattle (Garcia et al., 2008). Another change that occurred in 1997 was the USDA revised their standards for grades of

carcass beef which restricted Select to A maturity only and raised the marbling degree for Choice to minimum modest throughout B maturity (USDA, 1997) thereby increasing the amount of cattle grading US Choice.

Another change since 1986 includes the development of new technologies that have had an impact on the beef industry. Instrument grading has recently been accepted and has the ability to determine USDA quality and yield grades of beef carcasses (Woerner and Belk, 2008). Currently some packing companies have implemented camera grading into their facilities. One company using the E + V Vision Grading System collects over 40,000 images daily (Lorenzen, 2008). Some of the major advantages of this technology include enhanced grading accuracy and consistency, improved producer and packer confidence in grades and increased efficiency in the work place (Lorenzen, 2008).

In addition to new grading technologies there has been an increase of Association of Official Analytical Chemists (**AOAC**) approved new methods for total crude fat analysis. Ether extractable fat is still considered the gold standard method for fat extraction and was the only method used by Savell et al. (1986) in determining total fat percentages within marbling scores. Other methods include chloroform/methanol (**Folch**) extraction and, more recently approved by AOAC as a first action official method 2008.06, rapid determination of moisture and fat in meats by microwave drying and nuclear magnetic resonance (**NMR**). The method developed by Folch uses 2:1 ratio of extraction solvents chloroform:methanol (Folch et al., 1957). Folch is most commonly used in fatty acid analysis but can also be used to determine total fat percentage and can acquire results much faster than ether extraction. Association of Official Analytical Chemists method 2008.06 uses the CEM SMART Trac system which uses NMR to

determine total lipid content based on low-resolution time-domain NMR (Akoh and Min, 2002; Keeton et al., 2003).

The CEM SMART Trac system can obtain results within 10 min. The CEM is safe, fast, and reliable for determining moisture and total lipid content in raw and processed meat products (Keeton et al., 2003; Leefer et al., 2008) making it ideal for use in the meat industry. Ether is highly flammable and chloroform/methanol is not environmentally friendly and all these chemicals require proper disposal. The CEM SMART Trac system has more recently been compared to ether extraction. Research has shown that the CEM system is comparable to the AOAC method 950.46 (microwave drying) and 960.39 (Soxhlet ether extraction; Keeton et al., 2003; Leefer et al., 2008) but it has never been compared to the Folch method.

Determining total fat within meat is important because the industry continues to be scrutinized by the medical community about the healthiness of animal fat in the human diet. Food from animal sources are said to have high amounts of fats, mostly saturated fats and cholesterol, which are highly associated with high blood cholesterol and calories (NCEP, 2000). The American Heart Association's Dietary Guidelines and *Window of Acceptability* (Savell and Cross, 1988) suggest that the highest amount of fat that should be present in meat cuts is 7.3% for it to be a palatable and healthy part of the diet.

Determining the amount of fat within marbling scores can serve as a basis for nutritional labeling, confirming instrument grading, and determining genetic changes within the beef cattle population. Therefore, the objectives of this study were to determine the percentage of fat within USDA marbling scores for raw steaks, determine the relationship between three fat content methods in raw meat, and determine tenderness in relation to USDA marbling scores.

Chapter 2

LITERATURE REVIEW

2.1 Role of Fat in Meat Grading

2.1.1 History of Beef U.S. Quality Grading System. The first standards for U.S. beef grades were developed in 1916 and designed primarily for meat market reporting purposes. However, they were soon used to select beef for the war effort during World War I (USDA, 1997). Beef was selected based on the new grading system for the Army, Navy and Allied forces (USDA, 1997). Shortly after, the beef grading system was used to select beef by cruise ships, restaurants, hotels, fast food services and hospitals (USDA, 1997). In 1926 revised grading standards were made the Official United States Standards for the Grades of Carcass Beef (USDA, 1997).

Determining USDA quality grade takes into account the amount of intramuscular fat (marbling), size of marbling depots, distribution of marbling depots, lean color and fat color (Woerner and Belk, 2008). It is a hierarchal system based on marbling and maturity (USDA, 1997). As intramuscular fat in the ribeye increases marbling score increases which results in a higher quality grade (USDA, 1997). As the animal matures quality grade decreases (USDA, 1997).

Throughout the years the beef grading system has been amended several times and evolved to the system we use today (USDA, 1997). In 1965, official standards were revised to place less emphasis on changes in maturity in grades Prime, Choice, Good, and

Standard (USDA, 1997). The revision also included that all carcasses be ribbed prior to grading (USDA, 1997). In 1987 US Good grade was renamed to US Select (USDA, 1997). Then in 1997 another revision was made to restrict Select to A maturity carcasses only and Choice marbling degree was raised to minimum modest throughout B maturity carcasses (USDA, 1997). All of the revisions made were to improve the uniformity and consistency throughout the grades (USDA, 1997).

2.2 Differences in Marbling Scores

2.2.1 Effects of Marbling on Beef Tenderness. There are four theories that may explain why marbling may be influencing tenderness (Savell and Cross, 1988). (1) Bite theory, which suggests that with more intramuscular fat present there is less bulk density (Savell and Cross, 1988). Also lipid has a lower shear force value when compared to protein which may portray tenderness (Savell and Cross, 1988). (2) Strain theory, which indicates a thinning of connective tissue walls surrounding fat deposits and thereby decreasing tough connective tissue (Savell and Cross, 1988). (3) Lubrication theory, muscle fibers are lubricated by intramuscular fat which allows for a more tender and juicier product (Savell and Cross, 1988). (4) Insurance theory, suggests that greater marbled steaks can withstand higher cooking temperatures and even if the meat is over cooked it will still be palatable (Savell and Cross, 1988).

Several studies have been preformed comparing marbling levels and tenderness of beef *longissimus* muscle. Many studies have shown that as the amount of marbling increases tenderness also increases (McBee and Wiles, 1967; Tatum et al., 1980; Smith et al., 1987; Wheeler et al., 1999). However an equal number of studies including the National Beef Tenderness Surveys (1991, 2000, and 2007) show that marbling does not

affect tenderness (Smith et al., 1984; Morgan et al., 1991; Brooks et al., 2000; Voges et al., 2007). When comparing USDA quality grade, studies show that USDA quality grade can only explain 30 percent of the variation in tenderness (Smith et al., 1987; Li et al., 1999).

One explanation as to why marbling does not affect tenderness can be explained by the high number of studies that result in a high proportion of tender steaks based on the thresholds established by Shackelford et al. (1991) and studies resulting in all steaks being highly rated by consumers (Brooks et al., 2000). Voges et al. (2007) also found no difference in tenderness among quality grades however they noted that the majority of steaks were reported as tender which could be a result of longer aging periods, more gradual chilling rates, and a greater focus on beef tenderness. Longer aging periods has been shown to affect tenderness within marbling score (Wheeler et al., 1999). Steaks aged for three days showed as marbling score increased tenderness also increased, however steaks aged for 14 days marbling score had no affect on tenderness (Wheeler et al., 1999).

2.2.2 Effects of Marbling on Beef Palatability. Palatability is commonly described as the following: tenderness, juiciness and flavor. Using trained sensory panelists Smith et al. (1984) found that two-thirds of the time as marbling increased palatability also increased for loin steaks. However, palatability was not affected by marbling for round steaks (Smith et al., 1984). An increase in marbling levels from Slight to Moderately Abundant had no effect on trained sensory panel ratings for tenderness, juiciness and flavor or Warner-Bratzler shear force (**WBSF**) values (Smith et al., 1984). In the same data set, Smith et al. (1987) also compared USDA quality grade on beef palatability and found that US Prime carcasses were more palatable than US

Choice which were more palatable than Good/Select. Even though these results are more favorable, Smith et al. (1987) reported that USDA quality grade predicted flavor, tenderness, and overall palatability of loin steaks with only 30 to 38% accuracy.

Results from the National Consumer Retail Beef Study showed that consumers could detect palatability differences due to marbling levels (Cross et al., 1986). The Beef Customer Satisfaction Studies showed that USDA quality grade influences consumer overall liking of top loin steaks (Neely et al., 1998; Lorenzen et al., 1999; 2003). Overall liking of top loin steaks increased as marbling level increased from Low Select to Top Choice (upper 2/3 US Choice; Neely et al., 1998; Lorenzen et al., 1999; Lorenzen et al., 2003). Wheeler et al. (1999) found that as USDA quality grade increased juiciness and flavor intensity ratings also increased. However, USDA quality grade did not have an affect palatability of top sirloin or top round steaks in the Beef Customer Satisfaction Studies (Neely et al., 1998, 1999; Savell et al., 1999; Lorenzen et al., 2003). The *longissimus dorsi* can be segmented by USDA quality grade but not other muscles (Lorenzen et al., 2003).

Degree of doneness and palatability of beef can also be affected by USDA quality grade. Wheeler et al. (1999) suggested that steaks cooked to a high degree of doneness will have greater palatability rankings for Top Choice (upper 2/3 US Choice) than Low Select. The idea that marbling levels affect beef cooked well done agrees with the insurance theory developed by Savell and Cross (1988). Overall consumers prefer to cook steaks at a higher degree of doneness (Lorenzen et al., 1999; Savell et al., 1999; Neely et al., 1999); therefore steaks with higher marbling scores should have a greater chance of a good eating experience than low marbled steaks.

There continues to be debate on the whether or not marbling alone affects palatability due to several other affecting factors. Some of these factors include the effect of steak, city, and USDA quality grade on palatability (Neely et al., 1998) as well as household differences like cooking method, personal preference, degree of doneness, added seasonings, and thresholds of tenderness, juiciness and flavor (Lorenzen et al., 2003).

2.2.3 Effects of Marbling on Consumer Perception of Beef. When it comes to meat and meat products consumers are mostly concerned with safety, tenderness, flavor, high quality, and health (Lorenzen, 2008). The Beef Customer Satisfaction papers look at the interactions between USDA quality grade, cut, city, degree of doneness and cooking method on consumer satisfaction. An interaction between USDA quality grade and cut showed that consumers prefer top loin steaks over top sirloin and top round steaks (Neely et al., 1998; Lorenzen et al., 2003). There was an interaction between USDA quality grade and cooking method (Savell et al., 1999) and between USDA quality grade and degree of doneness which showed that Top Choice steaks were given higher ratings regardless of degree of doneness than Low Choice and Select steaks (Neely et al., 1999).

USDA quality grade also has an effect on consumers' willingness to pay. Platter et al. (2005) found that consumers are willing to pay more for high quality and more tender strip loin steaks. Consumers gave Prime steaks a \$2.47/kg premium and Choice a \$0.89/kg premium over Select steaks (Platter et al., 2005). Killinger et al. (2004) conducted a study looking at consumer perception of different marbling levels but similar in tenderness. They found that steaks with high marbling levels (upper 2/3 US Choice) were more accepted than steaks with low marbling levels (US Select) and that consumers

were willing to pay more for highly marbled steaks than lower marbled steaks when tenderness was the same (Killinger et al., 2004). Similarly, a significant relationship between consumer acceptance and marbling score indicated that consumer acceptance increased approximately 10% for each marbling score increase showing a linear relationship over all marbling scores (Platter et al., 2003).

Consumer perception is not only affected by marbling score and tenderness but also health perception (Savell et al., 1987; Neely et al., 1998). While Choice and Prime are highly accepted by most consumers, Select is still rated very high in overall acceptance due to leanness and the perception that leaner is healthier (Cross et al., 1986; Savell and Cross, 1988).

2.2.4 Window of Acceptability. The *Window of Acceptability* is based on the overall palatability of loin and rib steaks and the amount of marbling in those steaks. Marbling percentages within grade were determined by Savell et al. (1986) using ether extraction. Overall palatability was determined by the National Consumer Retail Beef Study (Cross et al., 1986) who found that even though consumers could detect differences between Choice and Select, they still rated Select highly acceptable because of leanness and less waste of fatty cuts. By combining fat percent within grade and overall acceptability Savell and Cross (1988) were able to create the *Window of Acceptability*. The window ranges from a minimum of 3% to a maximum of 7.3% fat which resulted in the range of low Select to high Choice (Savell and Cross, 1988). Using the *Window of Acceptability* Savell and Cross concluded that Select steaks could be sold to consumers at retail stores (Savell and Cross, 1988).

The “overall palatability” line (Figure 2) was a result of overall consumer ratings of strip loin steaks at given marbling scores (Savell and Cross, 1988). The line indicating

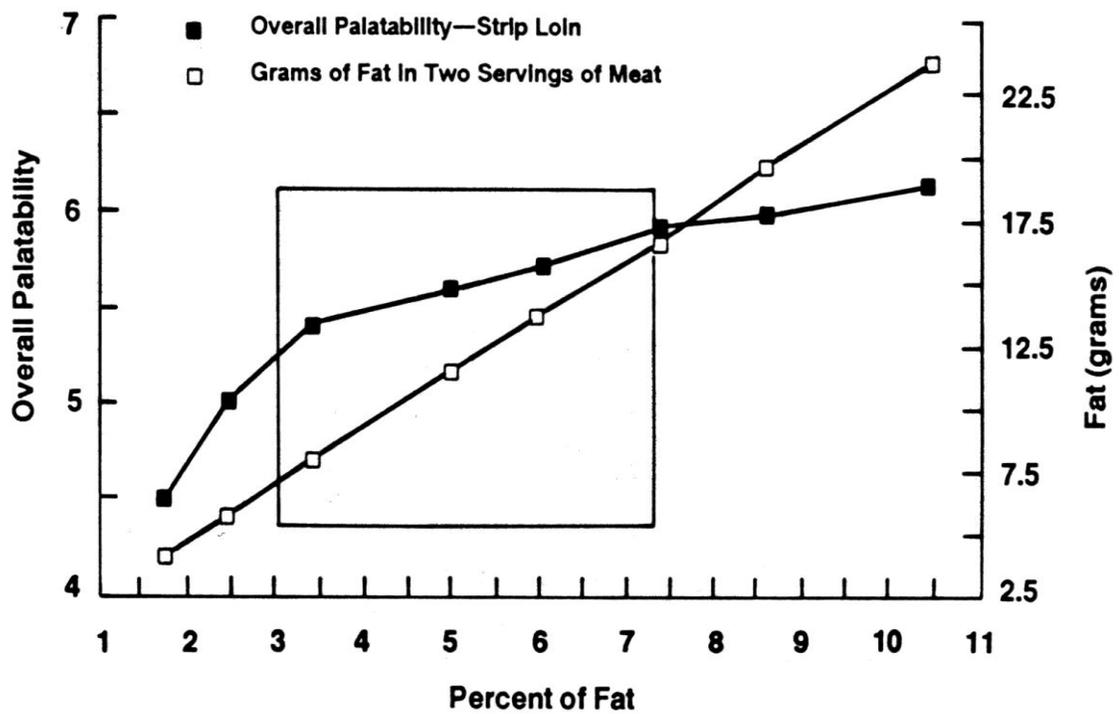


Figure 1. Window of Acceptability (Savell and Cross, 1988).

“grams of fat in two servings of meat” (Figure 2) was determined by extracting intramuscular fat using ether extractable fat within each marbling score (Savell et al., 1986). As the palatability line flattens out the window begins and once fat total reaches 7.3% the window ends giving an acceptable range for consumers to be from low Select to high Choice (Savell and Cross, 1988).

2.2.5 Effects of Beef Marbling on Nutrition. The American Heart Association (AHA; 2000) suggests eating lean meats, a limited amount of fatty meats, and a low amount of saturated fats. Animal food sources are said to have high amounts of fats, including saturated fats, and cholesterol, all of which have been highly associated with high blood cholesterol and caloric intake (NCEP, 2000). The *Window of Acceptability* and the American Heart Association’s Dietary Guidelines suggest the amount of fat present in meat cuts should not exceed 7.3% in order to decrease cholesterol level and lower the risk of heart disease (Savell and Cross, 1988; AHA, 2000; NCEP, 2000).

Since cholesterol is such a big concern there have been studies on whether or not animal fats affect cholesterol levels in humans. Rhee et al. (1982, 1988) found that there was no difference in cholesterol of cooked steaks with different marbling levels and consumers should not be concerned about marbling level of beef steaks. Distribution of cholesterol and fatty acid composition in muscle tissue is different among different muscles (Hoelscher et al., 1988; Turk and Smith, 2009). However total cholesterol did not differ among USDA quality grades for raw loin steaks (Hoelscher et al., 1988). For cooked loin steaks, Prime grade was higher than Choice or Select grades and Choice and Select grades were the same (Hoelscher et al., 1988). Chizzolini et al. (1999) suggests that intramuscular fat (marbling) only contributes to very low amounts of cholesterol.

Therefore consuming low marbling meat does not effectively reduce dietary cholesterol but it will reduce fat and caloric intake (Hoelscher et al., 1988; Chizzolini et al., 1999).

Hoelscher et al. (1988) also looked at the effect of fat trim on muscle tissue. They found that the cholesterol content of cooked steaks did not change with differing levels of subcutaneous fat trim (0.00, 0.64, and 1.27 cm) indicating that cholesterol from subcutaneous fat does not migrate into the lean meat (Hoelscher et al., 1988). Therefore, any fat trim left on the steak during cooking would not increase cholesterol levels in the same cooked steak (Hoelscher et al., 1988).

Research also shows that the presence of certain fatty acids in beef can lower plasma cholesterol levels. Stearic and oleic acid, which are present in intramuscular fat, lowered total plasma cholesterol by 15 to 20% (Bonanome and Grundy, 1988; Hassel et al., 1997).

2.3 Instrument Grading

Before the development of instrument grading beef grading has always been considered objective due to the extensive training of USDA graders. However, there is still variation among graders. The industry and the USDA discussed the need for a more objective grading system that would increase accuracy, precision, and speed (Cross and Whittaker, 1992). For the past 25 years instrument grading continues to develop in achieving these goals. The first type of technology designed for instrument grading was the video image analysis system (VIA) which was based on a camera/computer system (Cross and Whittaker, 1992). The system took measurements of muscle and fat areas, subcutaneous fat depth, and color of carcasses ribbed at the 12 and 13th rib and could measure up to 600 carcasses per hour (Cross and Whittaker, 1992).

With the development of the VIA system, the industry now had a more objective evaluation of carcass quality and yield grade traits (Cross et al., 1983). Initial testing of the VIA system was done at Kansas State University where Cross et al. (1983) concluded that the system had great potential, not only in quality grading but more so in determining yield grade. Cross et al. (1983) found in his research that the VIA system was a better predictor of rib composition than those developed from carcass traits measured by graders.

Unfortunately research with the VIA system was put on hold and more focus went toward ultrasound technology (Cross and Whittaker, 1992; Woerner and Belk, 2008). However, due to the lack of progress made by ultrasound technology, in 1994 priority and funding was directed toward VIA technology once again (Woerner and Belk, 2008). Recent research shows the VIA systems can be effective for cutability, marbling score, USDA yield grade and even predicting tenderness (Woerner and Belk, 2008). The VIA system has been shown to accurately determine yield grades at commercial chain speeds compared to USDA graders (Cannell et al., 2002; Shackelford et al., 2003; Steiner et al., 2003; Woerner and Belk, 2008). However, Shackelford et al. (2003) determined that the VIA system was not accurate enough in predicting marbling score for USDA quality grades.

Improvements were made and in 2006 the computer vision system (CVS) and the VBG2000 (E + V Technology) was developed (Woerner and Belk, 2008). This system was highly accurate and 98-99% repeatable and was approved in November 2006 (Woerner and Belk, 2008; Moore et al., 2010).

Currently some packing companies have implemented camera grading into their facilities (Lorenzen, 2008). One company using the E + V Vision Grading System

collects over 40,000 images daily (Lorenzen, 2008). Some of the major advantages of this technology include enhanced grading accuracy and consistency, improved producer and packer confidence in grades, and increased efficiency in the work place (Lorenzen, 2008). Instrument grading continues to provide a stronger foundation for beef market value (Lorenzen, 2008).

The approval process of instrument grading of beef carcasses outlined by the USDA (2006a, b) involves two stages: Performance Requirements for Instrument Marbling Evaluation I and II (**PRIME I** and **PRIME II**). There are two phases for **PRIME I**: phase I: Demonstration of the repeatability of marbling score prediction on stationary beef carcasses; and phase II: Demonstration of the accuracy and precision of marbling score prediction at line speeds (USDA, 2006a). According to USDA (2006a) requirements Phase I must be 95% repeatable and Phase II must meet the following for approval: average residual = 0 ± 10 marbling score units where the residual is the difference between the instrument marbling score and mean expert panel marbling score (MEPMS); the standard deviation of the residuals (rSD) from the MEPMS ≤ 35 marbling score units; and, slope of 0.000 ± 0.075 , using the residual from the MEPMS as the dependent variable (y-axis) and the average of the instrument marbling score and MEPMS as the independent variable (x-axis).

Performance Requirements for Instrument Marbling Evaluation II states that an establishment must have documentation of daily in-plant verifications and procedures that ensure accuracy and precision properly made by calibration and verification instruments that have been approved for marbling assessment (USDA, 2006b). Instruments for USDA grading have to meet both **PRIME I** and **PRIME II** requirements before

approval by the USDA (USDA, 2006b). PRIME I was approved November 2006 and PRIME II has yet to be approved (Woerner and Belk, 2008).

2.4 Lipid Components of Meat

There are three basic classifications of lipids: simple, compound, and derived (Hui, 2007). Simple lipids consist of nonpolar esters of fatty acids with alcohols (Hui, 2007). Compound lipids contain fatty acids with a compound addition such as a phosphate group, sphingosine, or carbohydrate (Hui, 2007). Derived lipids are not simple or compound but a mixed group of lipids such as free fatty acids, sterols, fat soluble vitamins, and hydrocarbons (Hui, 2007). These lipids are dispersed throughout the body in the form of kidney, pelvic and heart fat, subcutaneous fat, intermuscular fat, and intramuscular fat (marbling) (Aberle et al., 2001). Still other lipids contribute to cell membrane structure and function, hormones, and vitamins (Aberle et al., 2001).

The three types of lipids are triacylglycerides, phospholipids, and cholesterol. All cells in the body are surrounded by a fluid phospholipid bilayer (Aberle et al., 2001). They are found within the muscle tissue, surrounding muscle cells, nerve cells and blood vessels (Aberle et al., 2001). Hormones, derived from cholesterol, and fat soluble vitamins are also distributed throughout muscle tissues via blood vessels supplying energy and other metabolic functions (Aberle et al., 2001). Cholesterols account for a small percentage of total lipid content within different muscles (Hoelscher et al., 1988; Aberle et al., 2001). Most lipid content is present in adipose tissue depots associated with loose connective tissue between muscle bundles (Aberle et al., 2001). The total percentage of adipose tissue within the muscle can vary anywhere from 1.5 to 13% of which most are neutral lipids and phospholipids (Aberle et al., 2001). These lipids are

found in the form of triglycerides, long chain fatty acids with a glycerol backbone (Aberle et al., 2001). The most predominant fatty acids found in animal fats are palmitic, stearic, palmitoleic, oleic, linoleic, linolenic, and arachidonic (Aberle et al., 2001; Alfaia et al., 2009).

2.5 Fat Determination Method - Ether

2.5.1 Extraction. The gold standard for crude fat extraction is ether extraction using Association of Official Analytical Chemists (AOAC) Method 960.39 (2007). Petroleum ether or diethyl ether can be used as the extraction solvent using this method (Akoh and Min, 2002). Petroleum ether is less volatile compared to diethyl ether (Akoh and Min, 2002). It has a low boiling point (35 to 37°C) and contains mostly hexanes and pentanes (Akoh and Min, 2002). Petroleum ether is a nonpolar, hydrophobic solvent (Akoh and Min, 2002). Solvent extraction works by breaking van der Waals interactions, electrostatic interactions and hydrogen bonds (Akoh and Min, 2002). Neutral lipids then hydrophobically bond to the nonpolar solvent and are extracted from the sample (Akoh and Min, 2002). The amount of lipid extraction depends on the solubility of the different types of lipids present within each sample (Akoh and Min, 2002). Nonpolar solvents are extracting nonpolar lipids and vice versa (Akoh and Min, 2002).

2.5.2 Fat Components Extracted. Components that are extracted by ether solvents include nonpolar groups such as monoglycerides, diglycerides, and triacylglycerides (TAGs) (Akoh and Min, 2002). Other lipid compounds that are extracted are cholesterol, sterols, and glycolipids (Akoh and Min, 2002). The solubility of lipids increases with an increase in long chain fatty acids (Akoh and Min, 2002). Other compounds that can be extracted are lipid soluble vitamins, flavor compounds and

color compounds that are not desired when determining total lipid content (Akoh and Min, 2002).

Compounds that are not extracted by ether include phospholipids, free fatty acids, covalently bonded lipids and more polar short chain fatty acids (Akoh and Min, 2002).

2.5.3 Method Error. Error with ether extractable fat can be found throughout the procedure. Error begins with sample preparation. Particle size is important for lipid extraction with the smaller the particle size the more surface area is available for extraction (Akoh and Min, 2002). A representative sample size is needed to reduce error as well (Akoh and Min, 2002). Sample storage can also cause error (Akoh and Min, 2002). If samples are exposed to oxygen they are prone to oxidation of lipids which would result in under estimated lipid percentage (Akoh and Min, 2002). Freezing and thawing can cause exposure to enzymes that may result in lipid deterioration (Akoh and Min, 2002).

Error associated specifically with the ether procedure includes extraction time, drops per second and level of heat. Mandigo et al. (1967) found that error is reduced when using longer extraction time with samples containing high fat content of greater than 42%. For samples containing low fat levels extraction time had little effect on total lipid extraction (Mandigo et al., 1967). Drops per second and heat required for adequate extraction must be determined by each individual lab since each extraction apparatus and equipment are different (Mandigo et al., 1967).

2.6 Fat Determination Method - Folch

2.6.1 Extraction. The Folch method of extraction was developed by Folch et al. (1957). Folch uses a mixture of extraction solvents chloroform and methanol as a 2:1

ratio (Folch et al., 1957). This mixture of extraction solvents works the same way as ether in that they break van der Waals interactions, electrostatic interactions and hydrogen bonds and soluble lipids are extracted by the solvents (Akoh and Min, 2002). 2:1 chloroform and methanol is used in a two step extraction (Folch et al., 1957; Akoh and Min, 2002). The first step homogenizes the sample with the solvent mixture and then filtered, extracting about 95% of the total tissue lipids (Akoh and Min, 2002). The second step involves a water or salt solution wash. This wash separates the lipids in the lower phase while some highly polar lipids are lost in the upper phase (Akoh and Min, 2002).

2.6.2 Fat Components Extracted. The Folch solvent is a mixture of nonpolar chloroform and polar methanol (Akoh and Min, 2002). This mixture allows for a greater extraction of tissue lipids. Like ether, chloroform and methanol extracts neutral lipids such as TAGs and cholesterol (Akoh and Min, 2002). However, the Folch method, with more polar methanol will extract more polar lipids such as shorter chain fatty acids and some phospholipids such as diacylglycerophospholipids and sphingolipids (Akoh and Min, 2002). Highly polar lipids and all gangliosides are lost in the second step during the water wash (Akoh and Min, 2002).

Folch et al. (1957) stated that all tissue lipids other than strandin are contained in the lower phase. He also found that the addition of mineral salts in the washing step can help shift lipids from the upper phase to the lower phase while the mineral salts remain in the upper phase (Folch et al., 1957).

2.6.3 Method Error. Error is similar to ether in regards to sample preparation and sample storage. Sample size does not contribute to as much error as ether extraction because of the first step in homogenizing the sample with chloroform and

methanol. Error can occur if the sample is not given enough time to sit in chloroform/methanol solution before filtering and after washing. Samples need 30 minutes to sit before filtering and two hours to allow the two phases to separate (Folch et al., 1957). The greatest potential for error is found in the step where the upper phase is removed. If not all of the upper phase is removed then total lipid content will be overestimated and if some of the lower phase is removed then total lipid content will be underestimated.

2.7 Fat Determination Method - CEM

2.7.1 Extraction. The CEM SMART Trac system uses nuclear magnetic resonance (**NMR**) to determine total lipid content based on low-resolution time-domain NMR (Akoh and Min, 2002; Keeton et al., 2003). This system does not use any chemical solvents nor does it extract any components from the sample. Nuclear magnetic resonance uses magnetic field to detect radio frequencies admitted from hydrogen nuclei (H^+ or protons) of different food components are distinguished by each components different rates of decay or nuclear relaxation (Akoh and Min, 2002; Keeton et al., 2003). Liquid protons produce a slower signal which means they relax slowly or disappear last compared to other components in meat (Akoh and Min, 2002). Of those liquid components, lipids relax the slowest, giving off the last signal which is detected by the machine (Akoh and Min, 2002). Total fat content is determined by the intensity of the signal. Signal intensity is directly proportional to the number of lipid protons (Akoh and Min, 2002; Keeton et al., 2003).

2.7.2 Fat Components Extracted. The CEM SMART Trac can determine lipid, protein and carbohydrate content in food samples that have undergone microwave drying

(Keeton et al., 2003). The CEM SMART Trac system uses low-resolution NMR to detect the hydrogen nuclei of fat molecules in food samples (Keeton et al., 2003). Fat molecules longer relaxation times than other constituents and are detected last (Keeton et al., 2003). As fat within samples increases the signal intensity from the fat also increases and the computer determines total fat percentage (Keeton et al., 2003).

2.7.3 Method Error. Incorrect sample weights can have an adverse affect on both moisture and fat determination of the sample (Keeton et al., 2003). Samples that were greater than 5 g were not significantly affected, but it can cause the sample to burn during microwave drying (Keeton et al., 2003). Sample weight should range from 3 to 5 g to prevent this type of error (Keeton et al., 2003).

The temperature setting of the CEM can also have a negative effect on moisture and fat determination of the sample (Keeton et al., 2003). If the temperature is less than 125°C then sample may not be fully dried and moisture value will be under estimated, and if the temperature is greater than 125°C then there is potential for the sample to burn during drying (Keeton et al., 2003). Overheating samples can volatilize fat components resulting in a high moisture percentage (Keeton et al., 2003).

2.8 Comparing Fat Determination Methods

There is little research done comparing these different extraction methods and when these methods are compared even fewer determine the relationship between percent of total fat and USDA marbling score. In 1977 Hubbard et al. compared the Folch method, HCl digestion followed by ether extraction and a 2:2 chloroform and methanol solvent mixture for total lipid extraction of different foods. They found that the Folch method detected higher total fat when compared to the 2:2 ratio of chloroform and

methanol and that the Folch method was superior to acid digestion with ether extraction (Hubbard et al., 1977). The Folch method extracted the highest amount of sterols and cholesterol compared to the other methods (Hubbard et al., 1977).

In 1982, Rhee et al. used the Folch method to determine fat percent with different levels of marbling that were graded by professionally trained graders and found that marbling score was highly correlated with total lipid content. Steaks that were graded Practically Devoid had the lowest total fat percentage mean (2.37) while steaks graded as Moderately Abundant had the highest total fat percentage mean (12.08; Rhee et al., 1982). However, these means did not increase as marbling score increased. The total fat content of Slightly Abundant steaks were found to be the same as steaks graded as Small and steaks graded as Modest, Small and Slight were not significantly different (Rhee et al., 1982).

Four years later Savell et al. (1986) compared ether extractable fat with marbling score and, unlike Rhee, they found that as marbling score increased so did ether extractable fat percentage. They determined the regression equation for ether extractable fat percentage when marbling score is known is: Percent ether extractable fat = (marbling score x 0.0127) – 0.8043 with an R^2 of 0.7794 (Savell et al., 1986). Marbling scores were given numerical values as follows: Practically Devoid = 100, Traces = 200, Slight = 300, Small = 400, Modest = 500, Moderate = 600, Slightly Abundant = 700 and Moderately Abundant = 800 (Savell et al., 1986).

When comparing ether extractable fat to Folch extracted fat, Rhee et al. (1988) found that the Folch method extracts about 6.2% more fat than ether for raw samples and 13.4% more for cooked samples. Another paper comparing ether (soxhlet) and Folch extraction methods found Folch again extracted more total fats than ether (Perez-Palacios

et al., 2008). Based on these studies Folch is the recommended method for total lipid extraction (Hubbard et al., 1977; Rhee et al., 1988; Perez-Palacios et al., 2008).

The CEM SMART Trac system has more recently been compared to the AOAC approved ether method. Research has shown that the CEM system is comparable to the AOAC method 950.46 (microwave drying) and 960.39 (soxhlet ether extraction; Keeton et al., 2003; Leefer et al., 2008). It is also safe, fast and reliable for determining moisture and total lipid content in raw and processed meat products (Keeton et al., 2003; Leefer et al., 2008).

Hazardous chemicals are also important when comparing these three methods. The CEM SMART Trac does not require any hazardous chemicals for determining total fat, however the Folch method and ether extractable fat both use hazardous chemicals. Ether extractable fat uses petroleum ether which, according to Environmental Health and Safety (EHS, 2001) is highly flammable and can cause minor irritation. The Folch method uses chloroform which is also highly flammable, can cause moderate residual injury, and can be reactive at high temperatures (EHS, 2001). The Folch method also uses methanol which is moderately flammable (EHS, 2001). All of these chemicals must follow the proper disposable guidelines of unwanted hazardous materials (EHS, 2000).

Differences in fat determination methods are shown in Table 1.

Table 1. Comparison of fat determination methods.

	Fat Determination Methods		
	Ether ¹	Folch ²	CEM ³
Components Extracted	Extracts nonpolar lipids	Extracts more lipids (phospholipids)	Detects lipid proton signals
Fat Determination	Fat content is determined indirectly by weight	Fat content is determined directly by weight	Fat content is determined by signal intensity
Speed	Very slow	Slow	Fast
Risk Assessment	Uses hazardous chemicals	Uses hazardous chemicals	Safe; No hazardous chemicals

¹Petroleum ether.

²Chloroform/Methanol Solution.

³Microwave Drying and Nuclear Magnetic Resonance.

Chapter 3

DETERMINATION OF FAT PERCENTAGE USING THREE DIFFERENT METHODS WITHIN MARBLING SCORES ON BEEF LONGISSIMUS MUSCLE

3.1 Introduction

Marbling is the number one factor in valuing beef carcasses both by industry and consumers. Marbling scores are used to determine USDA quality grade for carcasses and pricing at the wholesale level with higher quality grades commanding higher prices. Consumers perceive marbling as a basis for estimating eating quality and nutritional value of beef steaks. Eating quality is a combination of tenderness, juiciness and flavor. An increase in USDA quality grade has been shown to increase flavor, tenderness and overall palatability (Smith et al., 1987).

In 1986, Savell et al. determined total fat percentage using ether-extractable fat within beef USDA marbling scores of *longissimus* muscle. Since 1986, cattle genetics and carcass traits have changed and there has been an increase of Association of Official Analytical Chemists (AOAC) approved methods for total crude fat analysis. Ether extractable fat is still considered the gold standard method for fat extraction and was the only method used by Savell et al. (1986) in determining total fat percentages within marbling scores. Other methods of fat extraction include chloroform/methanol and, more recently approved by AOAC as a first action official method 2008.06, rapid

determination of moisture and fat in meats by microwave and nuclear magnetic resonance (NMR).

The development of instrument grading has also had an impact on the beef industry. Instrument grading for determining USDA quality grades of beef carcasses was accepted in 2006 by the USDA (Woerner and Belk, 2008). Some of the major advantages of this technology include enhanced grading accuracy and consistency, improved producer and packer confidence in grades and increased efficiency in the work place (Lorenzen, 2008).

Determining the amount of fat within marbling scores can serve as a basis for nutritional labeling, confirming instrument grading, and determining genetic changes within the beef cattle population. Therefore, the objectives of this study were to determine the percentage of fat within USDA marbling scores for raw steaks, determine the relationship between three fat content methods in raw meat, and determine tenderness in relation to USDA marbling scores.

3.2 Materials and Methods

3.2.1 Sample Selection and Preparation

Samples (n = 119) were selected by USDA grading system (USDA, 1997) using an E + V Vision Grading camera at a commercial beef plant within a 12 h period (Table 2). One sample, approximately 5.08 cm thick, was cut from both sides of the carcass at the 13th rib, directly above the graded steak and transported to University of Missouri meat lab on ice. The sample from the right side of the carcass was allotted to Warner-

Table 2. Number of samples selected within marbling score.

Marbling Score	Marbling Degree within Marbling Score				
	0	20	40	60	80
Slightly Abundant	5	5	4	5	3
Moderate	4	5	5	5	4
Modest	5	5	4	5	5
Small	5	5	5	5	5
Slight	5	5	5	5	5

Bratzler shear force (**WBSF**) and the sample from the left side, which was graded by the camera, was allotted to fat extraction. All samples were aged for 14 d in the University of Missouri Meat Lab cooler (~2°C).

3.2.2 Warner-Bratzler Shear Force

Warner-Bratzler shear force was performed according to AMSA (1995) guidelines. WBSF samples were cut into 2.54 cm thick steaks, vacuum packaged, and aged for 14 d. Raw weight of each steak was recorded. A copper constantan thermocouple was placed in the geometric center of each sample and attached to an HH-21 calibrated thermometer. WBSF samples were cooked using a Hamilton Beach Portfolio Indoor/Outdoor Grill (Washington, NC) to an internal temperature of 35°C, flipped once and removed from the grill at 71°C. Final temperature was recorded. Cook weight of each steak was taken immediately after being removed from the grill. Steaks were then cooled at room temperature for 4 h. Six cores (1.27 cm diameter) were taken from each steak parallel to the muscle fibers. Cores were then sheared perpendicular to the muscle fibers using the United STM 'SMART-1' TEST SYSTEM SSTM-500 (United Calibration Corp., Hamilton Beach, CA). Settings include: force units (kg), linear units (mm), cycling (1 x 70 mm), test speed (250 mm/min), return speed (500 mm/min), and set up scales (CAP = 226.8). Shear force values for each core were determined using the computer. The mean WBSF values of the six cores were reported for each steak.

3.2.3 Fat Extraction Methods

Steaks allotted to fat extraction were trimmed of all external fat and twice ground using 8 mm and 4 mm grinding plates. The finely ground beef was mixed by hand and then split into three 200 ml plastic, pathology containers labeled for fat extraction. The

three methods used in fat extraction were 2:1 chloroform:methanol (**Folch**), microwave drying and NMR (**CEM**) using CEM SMART Trac system and ether extraction (**Ether**) using petroleum ether. Samples were frozen ($\sim -18^{\circ}\text{C}$) until extraction could be performed. Samples were thawed in refrigerator ($\sim 0^{\circ}\text{C}$) for 24 h prior to sample preparation. Samples were run in triplicate for each method. Samples not within three standard deviation of the mean were reprocessed.

Folch. This method was done according to Folch et al. (1957). One gram samples were weighed and placed in 10 mL centrifuge tubes. Approximately 5 mL of chloroform/methanol ($\text{CHCl}_3:\text{CH}_3\text{OH}$, 2:1, v:v) solution was added to the sample and homogenized with an OMNI International 2000 homogenizer (Waterbury, CT) for 30 sec. Samples were transferred to 50 mL centrifuge tubes. The 10 mL tubes were rinsed with chloroform/methanol solution until final volume of 50 mL tube was approximately 15 mL. To extract lipids, samples were allowed to sit under a hood for 30 min. The homogenized samples were then filtered through sintered glass filter funnel into a second 50 mL centrifuge tube. The homogenate was rinsed 2 to 3 times with chloroform/methanol solution. A volume of 8 mL KCl (0.74% KOH in methanol) was added to the filtered sample and vortex for approximately 30 sec. Samples sat for 2 h until two distinct phases appeared. The upper phase was carefully removed and discarded. The remaining sample was placed in a pre-weighed, 20 mL disposable scintillation vial and the 50 mL tube was rinsed 2 to 3 times with chloroform/methanol into the vial. The chloroform/methanol solution was evaporated to dryness with nitrogen using the Meyer N-Evap Analytical Evaporator (Organomation Associates INC, Berlin, MA). Extracted fat and glass vial were weighed and fat percentage was determined directly by weight.

CEM. This method was done according to Keeton et al. (2003). Using the CEM SMART Trac rapid fat analysis system two CEM spare sample pads were dried and 3.75 to 4.5 g of sample were smeared across one of the pads. The second pad was placed over the sample sandwiching the sample between both pads. Moisture percentage was determined by weight using the CEM Moisture/Solids Analyzer. The dried sample was then wrapped in TRAC paper and placed into a CEM TRAC tube and packed to the bottom of the tube. The tube was placed into the CEM Rapid Fat Analyzer. Fat percentage was determined on a dry basis using NMR and converted to wet basis.

Ether. Fat was extracted using AOAC Method 960.39 (2007) for ether extractable fat. Approximately 4 g samples were added to pre-weighed extraction thimbles, approximately 2.4 g of sand and a cotton ball size portion of glass wool. Samples were mixed with the sand using glass rods and the glass wool was used to cover the sample in the extraction thimble. Samples were dried in a 100°C drying oven for 24 h. Samples were then placed in a desiccator and cooled to room temperature at which time dry weight was recorded. Samples were then placed into a Labconco goldfish ether extraction apparatus (Serial # 65382, Kansas City, MO). Approximately 50 mL of petroleum ether (Cat. No. E139-20, Fisher Science, Fair Lawn, NJ) per sample was used for fat extraction. Ether was allowed to drip through samples for 6 h. Samples were removed and left to dry for 24 h. Samples were then dried at 100°C in drying oven for 90 min. Samples were again placed in a desiccator and allowed to cool to room temperature at which point weights were recorded. Fat percentage was determined indirectly by weight lost.

3.3 Statistical Analyses

Data was analyzed using SAS 9.1 (SAS Inst. Inc., Gary, NC). Means and standard deviations (SD) for carcass data were determined using the MEANS procedure. Data for percent extractable fat and tenderness were analyzed as a split plot in which degree of marbling score was the main plot and method and degree*method was the subplot. Mean differences were determined using PROC MIXED. Mean differences were determined using Fisher's least significant difference (LSD). A P value < 0.05 was considered significant.

Regression equations and slope differences were determined by analysis of covariance using the GLM procedure. The GLM procedure using Type I sum of squares was used to compare regression equations by method and by degree. Linear, quadratic and cubic polynomial orthogonal contrasts were performed in order to test differences between regression equations which indicated that at least two slopes were different (Table 3) over all marbling scores. When comparing slopes within each marbling score by method Type I sum of squares shows all methods had the same slope ($P > 0.05$). Therefore methods were pooled together to obtain regression equations for each marbling score using the REG procedure.

Table 3. Statistical analysis comparing linear, quadratic and cubic polynomial orthogonal contrasts analyzed by the GLM procedure using Type I sum of squares.

Source	Degrees of Freedom	Mean Square	<i>P</i> value
Method	2	56.50	< 0.0001
Degree	24	96.99	< 0.0001
Method*Degree	48	0.66	0.9952
Method*Degree Linear	2	3.74	0.0472
Method*Degree Quadratic	2	0.91	0.4743
Method*Degree Cubic	2	0.50	0.6650

3.4 Results and Discussion

In this study all samples used for fat extraction were graded by the E + V Vision Grading camera (VBG2000; E + V Technology) which was approved by USDA in 2006 meeting the Performance Requirements for Instrument Marbling Evaluation I (**PRIME I**; Woerner and Belk, 2008). In order to meet PRIME I standards the instrument demonstrated accuracy and precision of marbling score prediction and be over 95% repeatable (USDA, 2006a). Studies show that this system was highly accurate and over 98% repeatable at commercial production speeds (Woerner and Belk, 2008; Moore et al., 2010). Before instrument assessment of marbling score can be fully implemented it must meet both PRIME I and II requirements before approval by the USDA (USDA, 2006a, b). Performance Requirements for Instrument Marbling Evaluation II (**PRIME II**) states that an establishment must have documentation of daily in-plant verifications and procedures that ensure accuracy and precision properly made by calibration and verification instruments that have been approved for marbling assessment (USDA, 2006b). PRIME II has yet to be approved by the USDA.

Recent studies have shown that instrument grading and expert USDA graders were in agreement with each other (Shiranita et al., 1999; Woerner and Belk, 2008), therefore comparisons between this study and other studies which only used USDA graders were made.

3.4.1 Carcass Traits

Carcass traits of cattle selected for this study are shown in Table 4. Compared to the National Beef Quality Audit (**NBQA**; Garcia et al., 2008) the cattle in this study were

Table 4. Means, SD, and minimum and maximum values for carcass traits and WBSF.

Traits	Mean	SD	Min	Max
USDA yield grade	3.3	0.8	1.3	5.4
Fat thickness, cm	1.1	0.4	0.2	2.3
HCW ^a	383.0	35.4	260.4	483.5
REA ^b	81.1	8.8	62.6	109.0
Marbling score ^c	533	143	300	780
Final Temperature (°C)	72.4	3.8	69.0	99.3
Cook Loss (%)	20.2	5.5	9.4	36.4
WBSF ^d	30.4	6.9	19.6	57.9

^aHCW = hot carcass weight (kg)

^bREA = rib eye area (cm²)

^c300 = Slight⁰⁰, 400 = Small⁰⁰, 500 = Modest⁰⁰, 600 = Moderate⁰⁰, 700 = Slightly Abundant⁰⁰.

^dWBSF = Warner Bratzler shear force (N)

heavier, lighter muscled, had less fat but higher numerical USDA yield grades than the average population. The reason for these differences was due to sample selection. The NBQA was a random sample of the population as opposed to directly selecting for specific marbling scores in a proportion skewed towards higher USDA quality grades. Compared to the NBQA, USDA yield grade was numerically higher in this study which was also due to the skewed selection towards higher quality grades because as quality grade increases, yield grade also increases (Garcia et al., 2008).

3.4.2 Tenderness

Warner-Bratzler shear values were not different regardless of marbling scores ($P > 0.05$; Table 5). These results agree with Smith et al. (1984) who found that marbling score did not affect WBSF values. A possible explanation for these results may be due to the high number of tender beef animals. According to Shackelford et al. (1991) a WBSF value of 45.1 N or lower was rated by consumers to be “slightly tender”. Of our sample population 96.6% (data not presented in tabular form) had WBSF values of less than 45.1 N indicating that almost all of our sample population would be considered tender. It is more difficult to find differences in tenderness when there is a lack of variation in WBSF values in the population.

Means for WBSF within USDA quality grades are shown in Table 6. There were no differences in WBSF due to quality grade ($P > 0.05$). The National Beef Tenderness Surveys also found that USDA quality grade fails to explain the variation of WBSF values (Morgan et al., 1991; Brooks et al., 2000; Voges et al., 2007). On the contrary Smith et al. (1987) found that as USDA quality grade increased WBSF values decreased and sensory panel ratings increased indicating that higher quality grades were more

Table 5. Least squares means (SD) for extractable fat methods and means for Warner-Bratzler shear force (WBSF) of strip loin steaks within marbling score.

Marbling Score ¹	n	WBSF (N)	Extractable Fat Methods (% fat)		
			CEM	Ether	Folch
SLAB	22	28.4 (9.4)	9.18 ^{a,y} (1.75)	10.16 ^{a,x} (1.27)	10.90 ^{a,x} (1.97)
MD	23	27.5 (3.7)	6.83 ^{b,z} (1.41)	7.84 ^{b,y} (1.24)	8.78 ^{b,x} (1.52)
MT	24	29.4 (7.4)	4.92 ^{c,y} (0.95)	6.04 ^{c,x} (1.07)	6.33 ^{c,x} (1.37)
SM	25	33.3 (5.8)	3.66 ^{d,y} (0.76)	4.48 ^{d,x} (0.95)	4.67 ^{d,x} (1.09)
SL	25	30.4 (6.9)	2.52 ^{e,z} (0.71)	3.09 ^{e,y} (0.92)	3.41 ^{e,x} (1.02)

^{abcde}Means within a column lacking a common subscript letter differ ($P < 0.05$).

^{xyz}Means within a row lacking a common superscript letter differ ($P < 0.05$).

¹SLAB = Slightly Abundant, MD = Moderate, MT = Modest, SM = Small, SL = Slight.

Table 6. Least squares means (SD) for Warner-Bratzler shear force (WBSF) within USDA quality grades for strip loin steaks.

USDA Quality Grade	n	WBSF (N)
Prime	22	28.6 (6.9)
Choice	72	30.3 (6.2)
Select	25	30.4 (9.4)

tender. However, since 1987 there has been a greater focus on meat tenderness and the majority of steaks in recent studies have been reported as tender (WBSF < 45.1 N) which could be a result of longer aging periods and more gradual chilling rates (Voges et al., 2007).

3.4.3 Crude Fat

Fat percentage increased as marbling score increased ($P < 0.05$) regardless of fat extraction method (Table 5). These results agree with previous research where Ether was used and indicated that fat percentage increased with an increase in marbling scores (McBee and Wiles, 1967; Savell et al., 1986; Moore et al., 2010). High, positive correlations between marbling score and fat percentage were also observed in previous research (McBee and Wiles, 1967; Armbruster et al., 1983; Campion et al., 1975; Moore et al., 2010). As marbling score increased variation seemed to increase as well. Moore et al. (2010) reported that both expert panel graders and camera grading precision decreased as marbling score increased.

In this study, least squares means of Ether extractable fat were numerically higher for Slightly Abundant (**SLAB**), Moderate (**MD**), and Modest (**MT**) and lower for Small (**SM**) and Slight (**SL**) compared to Savell et al (1986). Compared to Moore et al. (2010), means for Ether extractable fat in this study were numerically higher for SLAB, MD, and SL and lower for MT and SM. Garcia et al. (2006) reported the same results using microwave drying and NMR with the CEM SMART Trac for determining percent fat within *longissimus* muscle. Means for CEM fat were numerically lower for all marbling levels in this study compared to Garcia et al. (2006) which may be due to sample size differences. Overall, higher marbling scores were expected to result in higher fat

percentages since marbling score is based mainly on the amount of intramuscular fat present within the *longissimus* muscle.

When comparing methods, Folch and Ether extracted a higher percentage of fat as marbling score increased than CEM for marbling scores SM, MT, and SLAB ($P < 0.05$; Table 5). All three methods extracted different amounts of fat at SL and MD marbling scores with Folch having the highest fat percentage and CEM having the lowest fat percentage ($P < 0.05$). Overall Folch extracted more fat than CEM ($P < 0.05$). Statistically Folch and Ether extracted the same amount of fat in this study however, previous research has shown that Folch extracts, on average numerically, 6.2% more fat than Ether (Rhee et al., 1988) where in this study Folch extracted 6.9% more fat than Ether which was slightly greater than Rhee et al. (1988). Another study compared the Folch method to HCl digestion followed by ethyl ether extraction and results indicated that the Folch method was superior to acid digestion with ether extraction (Hubbard et al., 1977).

The Folch solvent is a mixture of two parts nonpolar chloroform and one part polar methanol (Akoh and Min, 2002). This solution allows for a greater extraction of tissue lipids. Like Ether, chloroform and methanol extracts neutral lipids such as triacylglycerides (TAGs) and cholesterols (Akoh and Min, 2002). However, unlike Ether, the Folch method also extracts phospholipids (Akoh and Min, 2002) which Ether and CEM do not detect. The polarity of methanol extracts more polar lipids including phospholipids and shorter chain fatty acids (Akoh and Min, 2002). Ether can also extract lipid soluble vitamins, flavor compounds, and color compounds that are not desired when determining total lipid content (Akoh and Min, 2002). The CEM uses NMR to detect radio frequencies admitted from hydrogen nuclei (H^+ or protons) of different food

components (Akoh and Min, 2002; Keeton et al., 2003). Lipids give off the slowest signal compared to all other food components (Akoh and Min, 2002). Total fat percentage is determined by the intensity of the signal using the CEM computer. Signal intensity is directly proportional to the number of lipid protons (Akoh and Min, 2002; Keeton et al., 2003).

In this study Folch and Ether were shown to extract more fat than CEM ($P < 0.05$; Table 5). The CEM SMART Trac system has more recently been compared to the AOAC approved Ether method. Research shows that the CEM system is comparable to Ether (Keeton et al., 2003; Leefer et al., 2008) which does not agree with the results of this study. Possible explanations for these differences may be due to sample size differences and product differences. This study had a higher sample size and only compared beef samples where as Keeton et al. (2003) and Leefer et al. (2008) had a smaller sample sizes and compared different meat products such as beef, pork, and poultry.

3.4.4 Prediction Equations for Fat Determination Methods

Quality grade is determined based on marbling score and maturity. In this study all animals were of A maturity. Marbling score is based not only on the amount of intramuscular fat but also how the fat is distributed and the color of the lean. All fat determination methods in this study were determining total fat within the sample which did not take into consideration how the marbling was distributed nor the color of the lean. This may explain variation among marbling scores. Therefore, some samples with high fat percentages may have had fat that was not evenly distributed and thereby given a lower marbling score. Steaks with even marbling distribution are graded at a higher value than those that have uneven marbling distribution.

Prediction equations for all fat percentage determination methods were linear (Figure 2). The regression equation for CEM fat percentage = $-3.46 + 0.016$ (marbling score), with an R^2 of 0.824 ($P < 0.001$). Folch regression equation for fat percentage = $-3.42 + 0.019$ (marbling score), with an R^2 of 0.816 ($P < 0.001$). Ether regression equation for fat percentage = $-3.08 + 0.017$ (marbling score), with an R^2 of 0.859 ($P < 0.001$). Savell et al. (1986) found an Ether regression equation for fat percentage = $-0.8043 + 0.0127$ (marbling score), with an R^2 of 0.7794. Garcia et al. (2006) reported a CEM equation for crude fat = $-0.58 + 0.17$ (marbling score) with an R^2 of 0.55 and crude fat = $3.47 + 0.73$ (objective marbling score) with an R^2 of 0.54. When comparing equations, both Savell et al. (1986) and Garcia et al. (2006) reported lower R^2 values than found in this study indicating that the equation found in this study was slightly more accurate. Savell et al. (1986) used USDA graders to determine marbling scores. Garcia et al. (2006) used trained panelists and a computer scanning system to measure marbling score. In this study, only camera grading was used to determine marbling score. These differences in marbling score determination and differences in sample size may explain the variation between regression equations. Variation may also be caused by differences in sample selection. In this study we selected for specific marbling scores compared to Garcia et al. (2006) who selected for quality grades and Savell et al. (1986) who showed a skewed selection based on population.

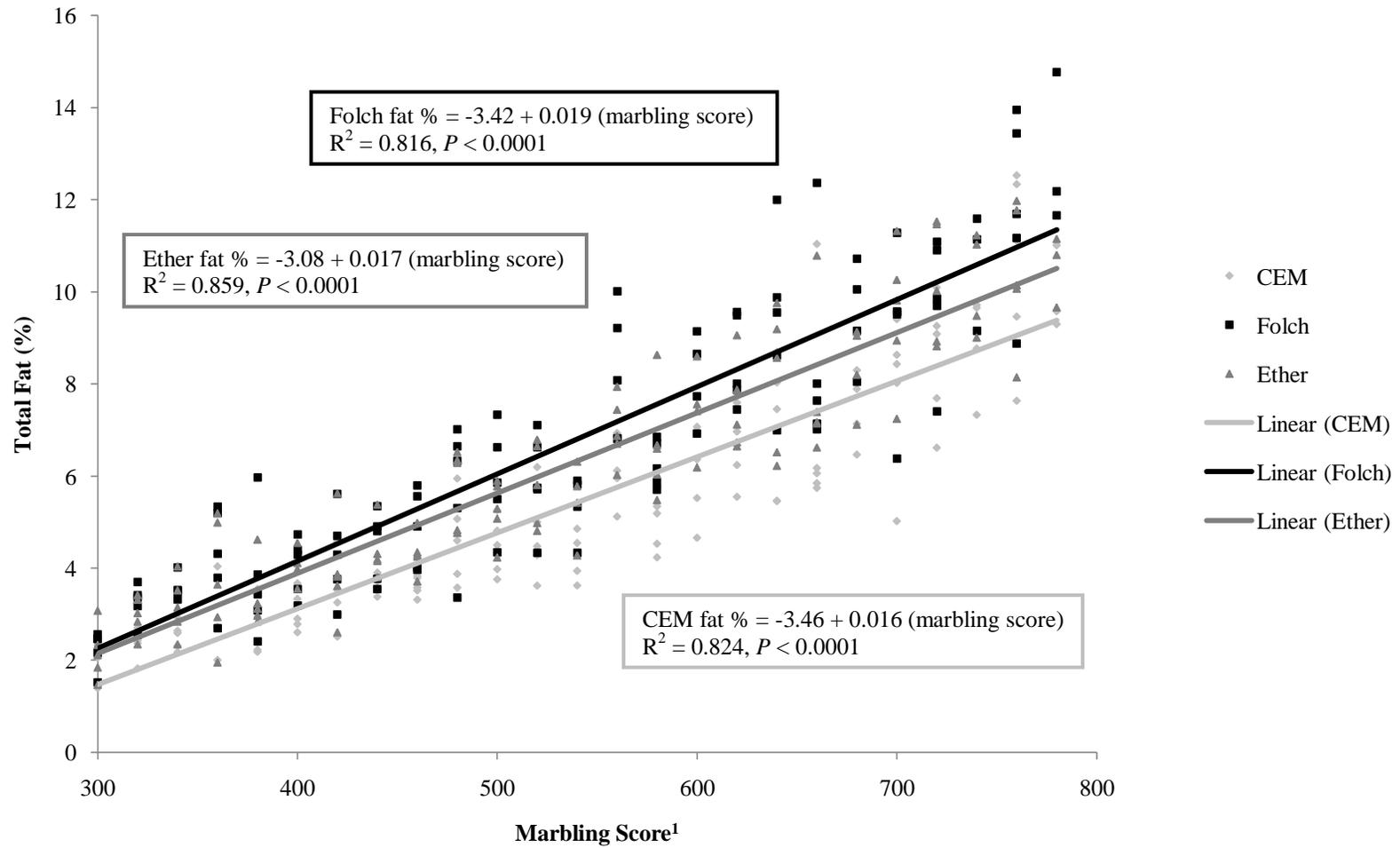


Figure 2. Regression Equations for Folch, Ether, and CEM fat determination methods of beef *longissimus* muscle. ¹ 300 = Slight⁰⁰, 400 = Small⁰⁰, 500 = Modest⁰⁰, 600 = Moderate⁰⁰, 700 = Slightly Abundant⁰⁰.

When comparing regression equations of all three methods the slope for CEM and Folch were different ($P < 0.05$). The Ether regression equation had the same slope as both CEM and Folch ($P > 0.05$). Therefore, as marbling score increased the Folch method extracted a higher percentage fat at a greater rate than CEM. These results may be explained by the greater variation of fat percentage at the higher marbling scores. Increased variation at higher marbling scores may be a result of camera error, in that the camera may not be detecting smaller flecks of fat that CEM, Folch, and Ether methods would detect. In addition, as the amount of fat within samples increases there is an increase potential for error. Another explanation may be that a higher genetic potential of the individual animal would increase the growth curve for fat, thereby accelerating fat build up at a greater rate than another individual which could also explain the variation of fat percentage at the higher marbling scores.

Regardless of these factors, the results suggest that any of the three methods, CEM, Folch, or Ether, can be used to determine total fat percentage. The equations will not change the grading system or the result of marbling score. In addition the prediction equations can be used to verify the camera grading system by determining fat percentage using any of the fat determination methods above and solving for the marbling score.

3.4.5 Prediction Equations within Marbling Scores

Regression equations within each marbling score were not different ($P > 0.05$) between methods. Therefore data from all three methods were pooled together to determine overall regression equations within each marbling score. Equations were as follows: SL fat percentage = $-2.682 + 0.0167$ (marbling score) with an R^2 of 0.2481 ($P < 0.001$; Figure 3). SM fat percentage = $-3.9151 + 0.0186$ (marbling score) with an R^2 of

0.2669 ($P < 0.001$; Figure 4). MT fat percentage = $-2.7976 + 0.0159$ (marbling score) with an R^2 of 0.1295 ($P < 0.05$; Figure 5). MD fat percentage = $-0.5697 + 0.0131$ (marbling score) with an R^2 of 0.0505 ($P > 0.05$; Figure 6). SLAB fat percentage = $-12.358 + 0.0304$ (marbling score) with an R^2 of 0.2191 ($P < 0.001$; Figure 7).

All equations were linear except MD which was a flat line. With low R^2 values these equations are very weak and show a lot of variation. Figures 3 to 7 show confidence bands and the amount of variation within each marbling score. With the amount of variation present these prediction equations are not suitable for proper nutritional labeling of fat percent within *longissimus* steaks. Some causes for variation may be low sample size and lack of marbling degrees.

In addition, marbling levels are not evenly distributed throughout the *longissimus* muscle (Cook et al., 1964). Therefore some samples may have had a higher fat level than predicted from the cut surface which would result in an under estimated marbling score and vice versa. Marbling score is a visual measurement and may not be conducive for determining a numerical value for total fat. Further research in this area is needed for predicting fat percentage within marbling scores.

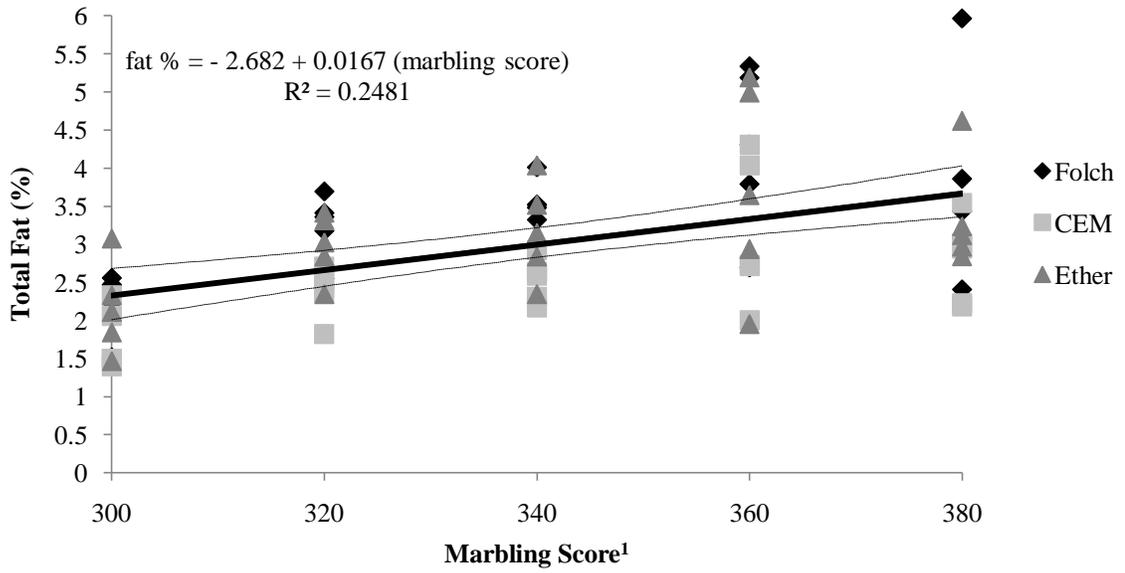


Figure 3. Regression equation for all fat determination methods at Slight marbling score of beef *longissimus* muscle. ¹300 = Slight⁰⁰, 320 = Slight²⁰, 340 = Slight⁴⁰, 360 = Slight⁶⁰, 380 = Slight⁸⁰. $P < 0.0001$

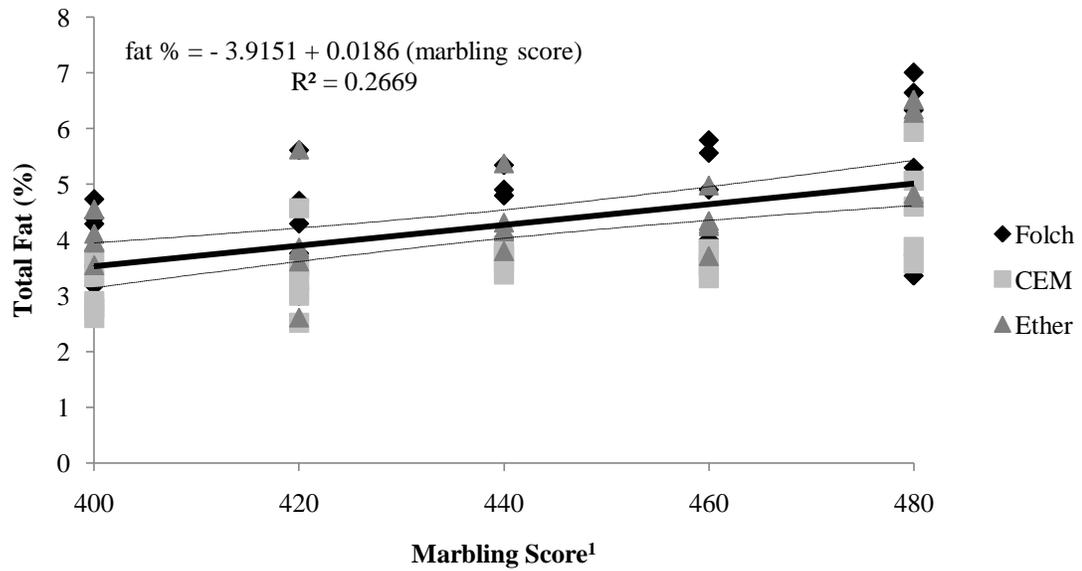


Figure 4. Regression equation for all fat determination methods at Small marbling score of beef *longissimus* muscle. ¹400 = Small⁰⁰, 420 = Small²⁰, 440 = Small⁴⁰, 460 = Small⁶⁰, 480 = Small⁸⁰. *P* < 0.0001

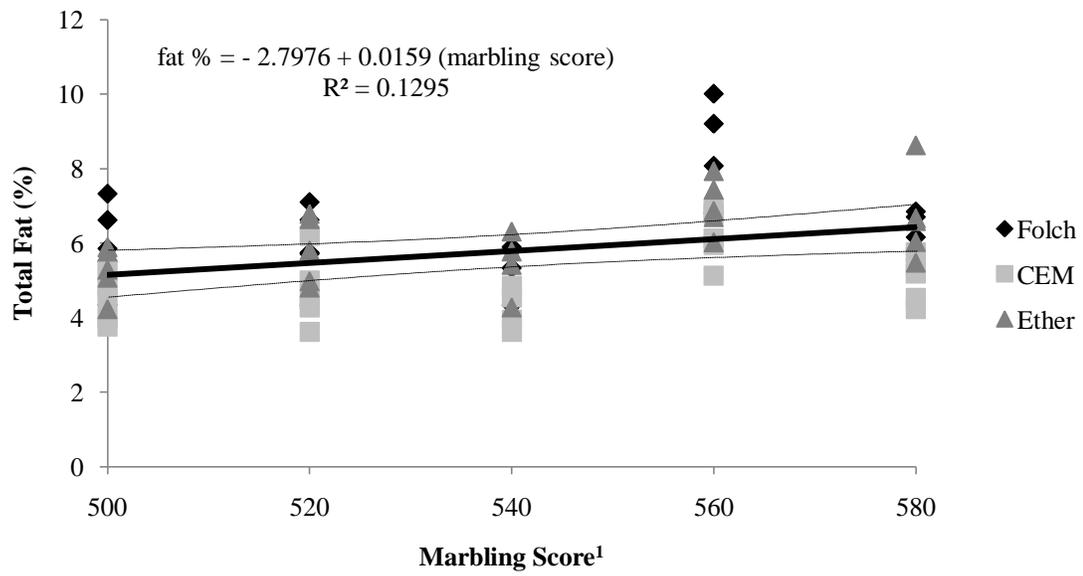


Figure 5. Regression equation for all fat determination methods at Modest marbling score of beef *longissimus* muscle. ¹500 = Modest⁰⁰, 520 = Modest²⁰, 540 = Modest⁴⁰, 560 = Modest⁶⁰, 580 = Modest⁸⁰. *P* = 0.0019

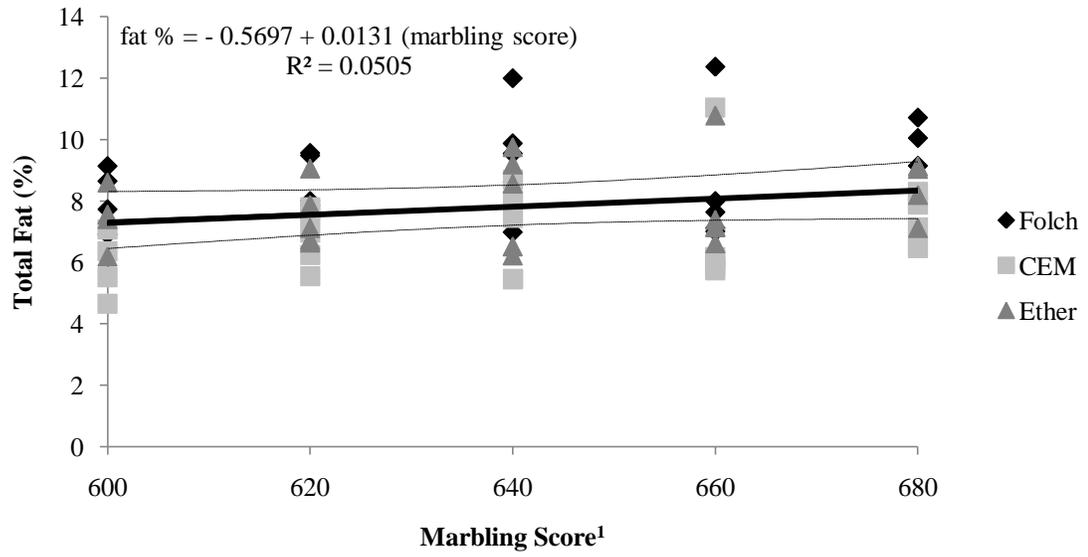


Figure 6. Regression equation for all fat determination methods at Moderate marbling score of beef *longissimus* muscle. ¹600 = Moderate⁰⁰, 620 = Moderate²⁰, 640 = Moderate⁴⁰, 660 = Moderate⁶⁰, 680 = Moderate⁸⁰. *P* = 0.0633

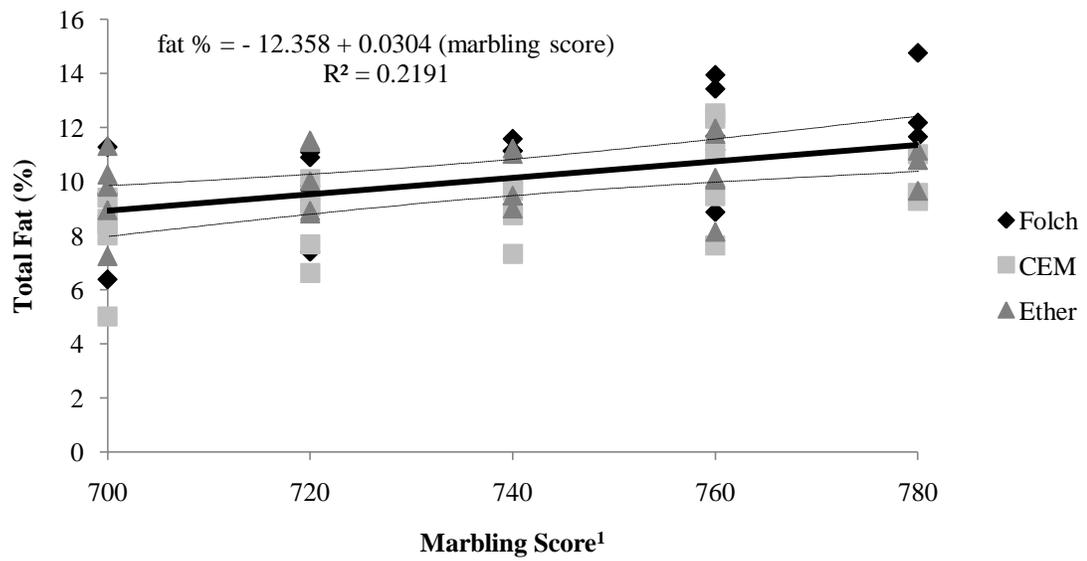


Figure 7. Regression equation for all fat determination methods at Slightly Abundant marbling score of beef *longissimus* muscle. ¹700 = Slightly Abundant⁰⁰, 720 = Slightly Abundant²⁰, 740 = Slightly Abundant⁴⁰, 760 = Slightly Abundant⁶⁰, 780 = Slightly Abundant⁸⁰. $P < 0.0001$

3.5 Conclusion

Tenderness was not affected by USDA quality grade or marbling score. Therefore marbling may not be the best indicator of tenderness. As marbling score increased total fat percentage also increased. The Folch and Ether methods extracted the same amount of fat and both extracted more fat than CEM. Folch extracts the greatest amount of fat numerically however Folch may be overestimating crude fat because it extracts the phospholipid bilayer that surrounds all cells. The prediction equation for Folch indicated an increase in fat extraction as marbling score increased compared to CEM. The prediction equation for Ether was the same as both CEM and Folch. Ether is the most accurate method based on the R^2 value. However, CEM is recommended because it is environmentally safe and the fastest method for determining total crude fat percentage.

Prediction equations within marbling score showed high amounts of variation and low R^2 values. Therefore these equations are not suitable for proper nutritional labeling of fat percent of *longissimus* steaks.

APPENDIX A: MATERIALS & METHODS

A.1 Sample Collection Procedure

1. Carcasses (n = 119; Table 1) were selected by USDA grading system (USDA, 1997) using an E + V Vision Grading camera at a commercial beef plant during one day.
2. Samples, approximately 5.08 cm thick, were cut from both sides of the carcass at the 13th rib, directly above the graded steak.
3. The sample from the right side of the carcass was labeled and allotted to Warner-Bratzler shear force (**WBSF**) and placed into a ziplock bag.
4. The sample from the left side, which was graded by the camera, was labeled and allotted to fat extraction and placed into a ziplock bag.
5. Samples were transported to University of Missouri meat lab in four large coolers on ice.
6. Samples were aged for 14 d in the University of Missouri Meat Lab cooler (~2°C).

A.2 Warner-Bratzler Shear Force Procedure

1. Samples were sliced into 2.54 cm thick steaks using the meat lab slicer and then vacuum packaged.
2. Steaks were frozen in the meat lab freezer ($\sim -20^{\circ}\text{C}$) until Warner-Bratzler shear force could be performed.
3. Twenty five to thirty two steaks at a time were removed from the freezer, placed flat on meat lab trays and allowed to thaw in the cooler for 48 h.
4. After 48 h, steaks were transferred to the lab refrigerator ($\sim 0^{\circ}\text{C}$) on meat lab trays.
5. Raw weight (g) of each steak was taken using a Mettler Toledo PB1502-S digital scale and recorded.
6. A copper constantan thermocouple was placed in the geometric center of each sample and attached to an HH-21 calibrated thermometer.
7. Steaks were cooked using four Hamilton Beach Portfolio Indoor/Outdoor Grills (Washington, NC).
8. All four grills were preheated for 10 min at a setting of five.
9. Two steaks were cooked on each grill at a time.
10. Each steak was cooked to an internal temperature of 35°C , flipped once and removed from the grill at an internal temperature of 71°C .
11. Final temperature ($^{\circ}\text{C}$) and cook weight (g) was recorded for each steak.
12. Steaks were then cooled for 4 h on meat lab trays to reach room temperature ($\sim 20^{\circ}\text{C}$).

13. The tail end of each steak was cut off to determine the direction of the muscle fibers.
14. Six cores (1.27 cm diameter) were taken from each steak parallel to the muscle fibers using a hydraulic drill press.
15. Cores were then sheared perpendicular to the muscle fibers using the United STM 'SMART-1' TEST SYSTEM SSTM-500 (United Calibration Corp., Hamilton Beach, CA). Settings include: force units (kg), linear units (mm), cycling (1 x 70 mm), test speed (250 mm/min), return speed (500 mm/min), and set up scales (CAP = 226.8).
16. Core values were recorded to the nearest 10th of a kg.
17. The six core values were averaged and recorded for each steak.

A.3 Sample Preparation for Fat Extraction

1. Samples allotted to fat extraction were trimmed of all external fat.
2. Samples were vacuum packaged and frozen in the University of Missouri Meat Lab freezer (~ -20°C) until they were ready to be processed.
3. Samples were thawed in the University of Missouri Meat Lab cooler (~ 2°C) for 48 h.
4. Samples were ground first using a Cabela's meat grinder (Pragotrade U.S.A. Inc., Cleveland, OH) with an 8 mm grinding plate.
5. Samples were ground a second time through a 4 mm grinding plates.
6. The finely ground beef was mixed by hand, split into three fractions and placed into three labeled 200 mL plastic, pathology containers with lid for fat extraction.
7. The three methods used in fat extraction were 2:1 chloroform:methanol (**Folch**), microwave drying and nuclear magnetic resonance (**CEM**) using CEM smartTRAC system and ether extraction (**Ether**) using petroleum ether.
8. Samples were frozen in the lab freezer (~ -18°C) until extraction could be performed.
9. Samples were thawed in refrigerator (~ 0°C) for 24 h prior to sample preparation.
10. Samples were run in triplicate for each fat extraction method.

A.4 Folch Procedure

Modified method according to Folch et al., 1957

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *The Journal of Biological Chemistry*. 497-509.

Chloroform/Methanol Solution:

2 parts chloroform, FW = 119.38 (ACROS, New Jersey)

1 part methanol, FW = 32.04 (Fisher Science, Fair Lawn, New Jersey)

KCl Solution:

0.74% KCl, LOT # 072560, FW = 74.56 (Fisher Science, Fair Lawn, New Jersey)

Distilled De-ionized Water

Procedure:

1. One gram samples were weighed and placed in 10 mL centrifuge tubes.
2. Approximately 5 mL of chloroform/methanol ($\text{CHCl}_3:\text{CH}_3\text{OH}$, 2:1, v:v) solution was added to the sample and homogenized with an OMNI International 2000 homogenizer (Serial # 1075, Waterbury, CT) for 30 sec.
3. Homogenized samples were transferred to 50 mL centrifuge tubes.
4. The 10 mL tubes were rinsed with chloroform:methanol solution until final volume of 50 mL tube was approximately 15 mL.
5. Samples were allowed to sit under a Hamilton Vectaire fume hood (Two Rivers, WI) for 30 min to allow for lipid extraction.
6. The homogenized samples were then filtered through sintered glass filter funnel into a second 50 mL centrifuge tube.

7. The homogenate was rinsed 2 to 3 times with chloroform/methanol solution.
8. A volume of 8 mL potassium chloride (0.74% KOH in methanol) was added to the filtered sample and vortex for approximately 30 sec.
9. Samples were allowed to sit under a Hamilton Vectaire fume hood (Two Rivers, WI) for 2 h allowing for two distinct liquid phases to appear.
10. The upper phase was carefully removed using a disposable glass pipette with vacuum suction and properly discarded.
11. Each 20 mL disposable scintillation vial was weighed (g) and recorded for each sample.
12. The remaining sample was placed in the pre-weighed, 20 mL disposable scintillation vial.
13. The 50 mL tube was rinsed 2 to 3 times with chloroform/methanol into the vial.
14. The chloroform/methanol solution was evaporated to dryness with nitrogen using the Meyer N-Evap Analytical Evaporator (Organomation Associates INC, Berlin, MA).
15. Extracted fat and glass vials were weighed (g) and fat percentage was determined using the following equation:

$$((\text{weight of vial and fat} - \text{weight of vial}) / \text{initial weight of sample}) * 100 = \%$$

Total Extracted Fat

A.5 CEM Procedure

Method performed according to Keeton et al., 2003.

Keeton, J. T., B. S. Hafley, and S. M. Eddy. 2003. Rapid Determination of Moisture and Fat in Meats by Microwave and Nuclear Magnetic Resonance Analysis. *Journal of AOAC International*. 86:1193-1202.

1. Using the CEM smartTRAC set the method to 0-30 BEEF.
2. Two CEM square sample pads were placed on the balance pan and the tare button was pressed.
3. Approximately 3.75 to 4.5 g of sample were weighed using the balance pan and then the sample was smeared across one of the pads using a scoopula spatula.
4. The second pad was placed over the sample sandwiching the sample in between both pads.
5. The sample and pads were placed on the balance pan within the CEM Moisture/Solids Analyzer.
6. The start button was pressed and moisture percentage was determined by the CEM Moisture/Solids Analyzer.
7. The dried sample was then wrapped in CEM TRAC paper using the following procedure:
 - a. Pads with dried meat were placed on CEM TRAC paper.
 - b. Opposite corners were folded in so they overlapped slightly in the middle.
 - c. Starting at one corner, the sample was rolled up in the TRAC paper.
 - d. The rolled up sample was placed into a CEM TRAC plastic tube.
8. The sample was packed to the bottom of the tube using the TRAC and the tube was placed into the CEM Rapid Fat Analyzer.

9. The Ready button was pressed and then the Start button was pressed.
10. Fat percentage was determined using nuclear magnetic resonance and the computer gave a total fat percentage on a dry matter (DM) basis.
11. DM values were converted to wet matter (WM) values using the following equation:

$$((100 - \% \text{ moisture}) / 100) * \% \text{ fat DM} = \% \text{ fat WM}$$

A.6 Ether Procedure

According to AOAC Official Method 960.39 and 945.16A (2007) for ether extractable fat.

Association of Official Analytical Chemist. 2007. *Official Methods of Analysis*. AOAC International. Method 960.39.

1. Cellulose extraction thimbles were labeled and approximately 1/4 tablespoon of sand was added to each thimble.
2. One cellulose extraction thimble with sand and a cotton ball size portion of glass wool were weighed (g) together and recorded.
3. Approximately 4 g of sample were added to each extraction thimble with sand.
4. Samples were mixed with sand using 125 mm glass stirring rods (Fisherbrand, USA).
5. The stirring rod was wiped using the glass wool.
6. The glass wool was lightly packed into the extraction thimble with the glass stirring rod covering the sample and sand.
7. Weight (g) of thimble, wool, sand and meat was recorded.
8. Samples were dried in a 100°C Fisher Isotemp drying oven (200 Series, Model 255G) for 24 h.
9. Samples were placed into a Scienceware* Chemical-Resistant Vacuum Desiccator (Series 08-594-15) and cooled to room temperature (~ 20°C) at which time dry weight (g) pre-extraction was recorded.
10. Samples were placed into metal thimbles and placed into a Labconco goldfish ether extraction apparatus (Serial # 65382, Kansas City, MO).

11. Fifty mL of petroleum ether (Cat. No. E139-20, Fisher Science, Fair Lawn, NJ) per sample was used for fat extraction.
12. The heating pads were turned on and set to a temperature that allowed for a drip rate of 5 to 6 drips per sec.
13. Ether was allowed to drip through samples for 6 h.
14. Samples were removed from the ether extraction apparatus and left to dry overnight by the vent in the ether extraction room.
15. Samples were dried at 100°C in the drying oven for 90 min.
16. Samples were again placed in a desiccator and allowed to cool to room temperature (~ 20°C) at which point weights (g) were taken.
17. Fat percentage was determined indirectly by weight lost using the following equations:

Wt of thimble, wool and sand * DCF = Pre-extracted Dry Corrected Thimble Wt

Wt of thimble, wool and sand * EDCF = Extracted Dry Corrected Thimble Wt

Wt of thimble, wool, sand, meat - Wt of thimble, wool and sand = Wet Meat Wt

Dry Wt pre-extraction - Pre-extracted Dry Corrected Thimble Wt = Dry Meat Wt pre-extraction

Dry Wt post extraction - Extracted Dry Corrected Thimble Wt = Extracted Dry Meat Wt

Dry Meat Wt pre-extraction - Extracted Dry Meat Wt = Ether Extracted Fat Wt

(Ether Extract Fat Wt / Wet Meat Wt) * 100 = % Ether Extractable Fat on Wet Basis

Twelve blanks were run using the same procedure above without meat to determine dry thimble correction factor (DCF) and extracted dry thimble correction factor (EDCF).

DCF was determined using the following equations:

Dry Wt (g) pre-extraction/Wt (g) of thimble, wool and sand = DCF for each blank.

Average (DCF for each blank) = DCF

EDCF was determined using the following equations:

Dry Wt (g) post extraction/Wt (g) of thimble, wool and sand = EDCF for each blank

Average (EDCF for each blank) = EDCF

APPENDIX B: SAS PROGRAMS

B.1 Carcass Data

```
data one;
infile 'F:\csv files\carcassdata.csv' dsd firstobs=2 missover;
input mat$ carcass marbl$ mdegree rep HCW REA YG FT;
if marbl='SL' then m=300;
if marbl='SM' then m=400;
if marbl='MT' then m=500;
if marbl='MD' then m=600;
if marbl='SLAB' then m=700;
marbling=m+mdegree;
proc means;
run;
```

B.2 Cook Loss

```
data one;
infile 'F:\csv files\cookyield.csv' dsd firstobs=2 missover;
input grade steak rawwt temp cookwt cookloss;
cookloss=(rawwt - cookwt)/ rawwt *100;
proc means;
run;
```

B.3 Warner-Bratzler Shear Force

```
data one;
infile 'F:\csv files\dd.csv' dsd firstobs=2 missover;
input m1$ deg1 rep1 f1 w1 m2$ deg2 rep2 f2 w2 m3$ deg3 rep3 f3 w3;
data two; set one;
method=m1; degree=deg1; rep=rep1; fat=f1; wbsf=w1; output;
method=m2; degree=deg2; rep=rep2; fat=f2; wbsf=w2; output;
method=m3; degree=deg3; rep=rep3; fat=f3; wbsf=w3; output;
drop m1-m3 deg1-deg3 rep1-rep3 f1-f3 w1-w3;
data two; set two;
deg=degree/100;
deg=int(deg)*100;
proc mixed;
class method deg;
model wbsf=method deg method*deg;
lsmeans method | deg/pdiff;
```

B.4 Percent Fat Means within Marbling Scores

```
data one;
infile 'F:\csv files\new.csv dsd firstobs=2 missover;
input method$ degree rep fatavg anim;
deg=degree/100;
deg=int(deg)*100;
ID=anim;
proc mixed;
class method deg ID;
model fatavg=method|deg;
random ID(deg) id(method deg);
lsmeans method|degree/pdiff;
run;
```

B.5 Overall Percent Fat Means within each Fat Extraction Method

```
data one;
infile 'F:\csv files\new.csv dsd firstobs=2 missover;
input method$ degree rep fatavg anim;
deg=degree/100;
deg=int(deg)*100;
ID=anim;
proc glm;
class method degree;
model fatavg=method|degree;
lsmeans method|degree/pdiff;
run;
```

B.6 Linear, Quadratic and Cubic Polynomial Orthogonal Contrasts

```
data one;
infile 'F:\csv files\new.csv dsd firstobs=2 missover;
input method$ degree rep fatavg anim;
deg=degree/100;
deg=int(deg)*100;
ID=anim;
proc glm;
class method;
model fatavg=method degree degree*degree degree*degree*degree
method*degree method*degree*degree method*degree*degree*degree;
run;
```

B.7 Linear Regression Equations for Fat Extraction Methods

```
data one;
infile 'F:\csv files\new.csv dsd firstobs=2 missover;
input method$ degree rep fatavg anim;
deg=degree/100;
deg=int(deg)*100;
ID=anim;
proc glm; class method;
model fatavg=method degree*method/noint solution;
estimate 'i1-i2' method 1 -1 0;
estimate 'i1-i3' method 1 0 -1;
estimate 'i2-i3' method 0 1 -1;
estimate 'b1-b2' degree*method 1 -1 0;
estimate 'b1-b3' degree*method 1 0 -1;
estimate 'b2-b3' degree*method 0 1 -1;
run;
```

B.8 Linear Regression Equations within Marbling Scores

```
data one;
infile 'F:\csv files\new.csv dsd firstobs=2 missover;
input method$ degree rep fatavg anim;
deg=degree/100;
deg=int(deg)*100;
ID=anim;
proc sort; by deg;
proc glm; by deg;
model fatavg=method|degree;
lsmeans method|degree;
run;

proc glm; by deg;
class method; by deg;
model fatavg= method degree degree*degree degree*degree*degree
method*degree method*degree*degree method*degree*degree*degree;
run;

proc reg; by deg;
model fatavg=degree/r p;
run;
```

B.9 Confidence Lines within Marbling Scores

```
data one;
infile 'F:\csv files\new.csv' dsd firstobs=2 missover;
input method$ degree rep fatavg anim;
deg=degree/100;
deg=int(deg)*100;
ID=anim;
proc sort; by deg;
proc gplot; by deg;
plot fatavg*degree fatavg*degree/overlay;
symbol1 i=r1clm95
value=diamond;
run;
```

APPENDIX C: DATA COLLECTION SHEETS

C.1 Carcass Data

ANNO:				Carcass No:	
Marbling Level:		Marbling Degree:		Maturity:	
HCW:		REA:		YG:	
Fat Thickness:					

ANNO:				Carcass No:	
Marbling Level:		Marbling Degree:		Maturity:	
HCW:		REA:		YG:	
Fat Thickness:					

ANNO:				Carcass No:	
Marbling Level:		Marbling Degree:		Maturity:	
HCW:		REA:		YG:	
Fat Thickness:					

ANNO:				Carcass No:	
Marbling Level:		Marbling Degree:		Maturity:	
HCW:		REA:		YG:	
Fat Thickness:					

ANNO:				Carcass No:	
Marbling Level:		Marbling Degree:		Maturity:	
HCW:		REA:		YG:	
Fat Thickness:					

SL = Slight SM = Small MT = Modest MD = Moderate SLAB = Slightly Abundant

C.2 Warner-Bratzler Shear Force

Date: _____

Marbling Score						
Rep						
Raw Weight (g)						
Time On						
Final Temp (°C)						
Time Off						
Cook Weight (g)						
WBSF (kg)						
1						
2						
3						
4						
5						
6						
Ave WBSF						

*Cores cannot be taken for at least 4 hrs after the steak has been removed from the grill and the steak should be at room temperature.

Page No. _____

C.3 Ether

Date: _____

Sample	Thimble #	Wt (g) of thimble, wool & sand	Wt (g) of thimble, wool, sand & meat	Dry Wt (g) Pre-extraction	Dry Wt (g) Post extraction
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				

Date: _____

	13				
	14				
	15				
	16				
	17				
	18				
	19				
	20				
	21				
	22				
	23				
	24				

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VITA

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Danell was then accepted to California Polytechnic State University, San Luis Obispo, CA. While at Cal Poly she worked as a vet tech, a softball scorekeeper for the city of San Luis Obispo, and assisted in the Cal Poly meat lab. She received her B.S in Animal Science with a minor in both Meat Science and Agribusiness in 2008.

Upon graduating from Cal Poly she was accepted to the University of Missouri, Columbia to begin her Master's under Dr. Carol Lorenzen. Along with course work and research, Danell was also the laboratory instructor for Principals of Meat Science. She received her M. S. in Animal Science in July 2010.