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Effects of USDA quality grade and heating on palatability, cholesterol content, fatty acid and proximate composition of beef strip loin steaks

Robert Dean Johnson

Iowa State University

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Effects of USDA quality grade and heating on palatability, cholesterol content, fatty acid and proximate composition of beef strip loin steaks

Johnson, Robert Dean, Ph.D.

Iowa State University, 1993
Effects of USDA quality grade and heating on palatability, cholesterol content, fatty acid and proximate composition of beef strip loin steaks

by

Robert Dean Johnson

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Meat Science

Approved:
In Charge of Major Work
For the Major Department
For the Graduate College

Iowa State University
Ames, Iowa
1993
I would like to dedicate this dissertation to the memory of Milton Johnson and Fred Arett, my grandfathers, who enriched my life. Milton was a rancher, farmer from Gayville, SD and Fred was a dairy farmer from Austin, MN. They both enjoyed the simple pleasures that only rural life could offer. Milton was always very well-informed and politically active. He taught me to not be bashful and to "tell it like it is." Throughout this dissertation, striking examples can be found which support this premise. Fred was a very devoted, caring man, he loved his family, land and livestock. Again, the approach to which this investigation was conducted and articles written may trace back to this heritage.

Both men were afflicted with cardiovascular disease, to which they eventually succumbed. It is my intent that results from this investigation may be used in a manner which can help benefit society by making beef consumers more well-informed decision makers regarding nutrient composition of beef.
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GENERAL INTRODUCTION

USDA quality grades for beef were originally established to serve as guidelines to promote more systematic and uniform standards to classify carcass beef. These are used to facilitate marketing between various segments involved with the production, transformation and sale of beef. In the first official grade standards, no reference to palatability expectations were mentioned. However, as quality grading standards have evolved, definitions associated with quality now refer to the "palatability indicating characteristics of the lean." Formerly, retailers promoted beef on the basis of quality grade; namely USDA Choice. This was because of the perceptions of high eating satisfaction associated with beef from this grade. More recently, many retail markets are offering consumers more alternatives by positioning leaner cuts from the Select grade in self-service cases and even merchandising highly-marbled cuts from the Prime quality grade in full service showcases.

These moves have been a progressive step forward for a very traditional industry. Considering competitive processed meat items, variety, in terms of leanness and expectations related to palatability, has been the rule, rather than the exception. The cattle industry has spent considerable resources attempting to identify modern consumers and evaluate how beef can fit into their lifestyles. The impetus behind providing more lean beef cuts into the marketplace was the realization that society is more aware and concerned about health and the escalating cost of health care. Because certain health conditions may have association with and/or be controlled by diet, many consumers have altered their food preferences to reduce intake of total fat, saturated fat and cholesterol.
Furthermore, we know that variation in degree of marbling accounts for only about 10 to 25% of the variation in palatability characteristics from young beef. We also know that meat cookery has a significant effect on the palatability of beef. Previous research has shown that degree of doneness is more important to the palatability of the meat than is the degree of marbling. Therefore, cookery is an important factor in determining satisfaction derived from consuming beef cuts. Yet from a research viewpoint, cookery seems to have been down-played compared with studies conducted to investigate palatability differences related to quality grade, genetics, nutrition, and management.

Consequently, the purpose of our investigation was to determine if palatability could be optimized for strip loin steaks from different quality grades by broiling at different temperatures to different degrees of doneness. Furthermore, using steaks adjacent to those used for assessment of palatability, the effects of cookery on cholesterol content, fatty acid and proximate composition and calculated caloric density was assessed.

Explanation of Dissertation Format

This dissertation consists of a general introduction, a general review of literature, two different publishable papers, and a concluding general summary. All citations of references are in accordance with the CBE Style Manual used by the Journal of Animal Science for which these papers will be submitted. Each individual paper consists of an abstract, materials and methods, results and discussion, and an implications section. References cited in the general review of literature are listed following the general summary.
GENERAL REVIEW OF LITERATURE

General Background of Cholesterol and Methods to Assess Its Quantity

Cholesterol is the most highly decorated small molecule in biology. Thirteen Nobel Prizes have been awarded to scientists who devoted major portions of their careers to investigating cholesterol and implications associated therein. It was first isolated from gall stones in 1784 and since then has become a fascination for study in both science and medicine. Cholesterol is an interesting molecule in that the very property that makes it useful in cell membranes, chiefly its complete insolubility in water, also makes it lethal (Brown and Goldstein, 1985).

Cholesterol is characterized as a steroid alcohol with the chemical formula \( \text{C}_{27}\text{H}_{45}\text{OH} \). It is a biologically essential compound that functions in normal cell membrane activities by modulating the fluidity of the cell membrane. Furthermore, cholesterol is also the precursor of steroid hormones such as progesterone, testosterone, estradiol and cortisol along with bile acids and provitamin D3 (Sweeney and Weihrauch, 1976). Cholesterol, in addition to being stored as a membrane component, largely comprises tissues of the brain and spinal cord.

There are two sources of cholesterol for humans, dietary and self-synthesized. Average intake of cholesterol in the American diet is about 500 mg/day and provides about 200 mg/day of absorbed cholesterol at an absorption rate of 40%. Amount of dietary cholesterol absorbed is about 20% of the approximately 1 gm of cholesterol which is synthesized daily in the liver.
Appreciable amounts are synthesized by the intestine in adults (Olson, 1987). Rate of cholesterol formation by the liver and intestine is highly responsive to the amount of cholesterol absorbed from the diet. Feedback regulation is mediated by changes in the activity of 3-hydroxy 3-methyl-glutaryl CoA reductase. Dietary cholesterol suppresses the synthesis of the reductase in the liver and inactivates existing enzyme molecules (Stryer, 1988). Dietary cholesterol, however, does not account for more than 50% of the cholesterol synthesized per day (Siperstein et al., 1964). Also, cholesterol is metabolized to bile salts at a rate which amounts to about 50% of the sterols excreted in the stool under normal conditions (Olson, 1987). These bile salts are polar derivatives of cholesterol that act as highly effective detergents because they contain both polar and non-polar regions. These are synthesized in the liver, stored and concentrated in the gall bladder, and then released into the small intestines. Bile salts solubilization of dietary lipids increases the surface area which promotes hydrolysis by lipases and facilitates their absorption by the intestine (Stryer, 1988). Furthermore, small amounts of cholesterol (1%) are converted to vitamin D precursors and steroid hormones (Olson, 1987).

Although a vast amount of information has accumulated over the past century showing that diet plays some role in the pathogenesis of atherosclerosis and coronary heart disease, there is little evidence that dietary cholesterol is a major factor in initiating these conditions (Olson, 1987; McNamara et al., 1987). Of the risk factors usually listed for coronary heart disease, namely family history, hypercholesterolemia, hypertension, cigarette smoking, diabetes, obesity, age, sex and lack of exercise, none is diet predictable, and together account for only 50% of the risk of a person developing coronary heart disease.
The risk of the diet depends on its composition and upon the response of the host. Since both of these are variable, contribution of the diet to the incidence and mortality of coronary heart disease is not fully known, but it is expected to be less than 20% (Olson, 1987).

Despite the interest in cholesterol, limited information is available on the cholesterol content of foods, particularly those which require heat preparation before consumption. Although it is recognized that cholesterol is present and has been analyzed in foods primarily of animal origin such as beef, pork, lamb, domesticated fowl, fish, seafood and wild game, cholesterol contents have not been routinely analyzed by the most sensitive, selective, and precise methods available to researchers today. Laboratory technology has been greatly enhanced which has increased the ability to determine cholesterol content more accurately than in the past. Frequently, the range of cholesterol values previously reported in the scientific literature has been quite wide and a proper and thorough description of analytical methodology are often not provided which tends to make data interpretation difficult. Listing cholesterol content on nutritional labels which is proposed by the FDA and USDA will become mandatory in certain processed foods starting in July 1994. Certain cholesterol values reported in the literature may appear to be reasonably good, however, there is an urgent need for reinvestigation of the cholesterol content of foods by the more recently developed methods of analysis. Until this is done, many cholesterol values presently available can only be considered approximations.

Many of the procedures used for determining cholesterol in foods were originally developed for cholesterol in blood serum. Unfortunately, they were
often used for food without proper precautions to determine whether they were suitable for analytical measurement of particular foods.

From a historical point of view, modern cholesterol determinations trace their beginnings to the late 1800's when Salkowski described color reaction for cholesterol which was isolated from gall stones about a 100 years earlier. There were three studies of significance which helped propagate the movement for determination of cholesterol (Zak, 1977). These were the development of a color reaction, its modification and the discovery that digitonin precipitates cholesterol quantitatively. The combination of a color reaction for quantification and digitonide precipitation for purification led to the first colorimetric procedure for serum based on the gravimetric procedure of Windaus (1910). This was the first acceptable reference procedure. It was later used by Schoenheimer and Sperry (1934) to validate the accuracy of their procedure, and later it became the reference to validate the Abell et al., (1952) procedure. Both of these are now reference procedures. This indicates that present day accuracy is easier to accomplish, but is perhaps no better than that obtained by the Windaus (1910) gravimetric procedure. When considering the scientific press, literally hundreds of cholesterol methodology papers have been published, most being slight modifications of others (Zak, 1977).

In a comprehensive review, Sweeney and Weihrauch (1976) stated that extraction of lipids, separation of cholesterol from interfering substances and lipids, and detection and measurement are the steps necessary to determine cholesterol accurately and precisely.
Lipid Extraction

Cholesterol is extracted from biological materials with organic solvents. Several organic extracting solvents which have been tried include acetone, ethyl ether ethanol, acetone-absolute ethanol, ethanol ethyl ether, ethanol acetone, chloroform, absolute ethanol, methanol acetone, acetone-ethanol-ether 4:4:1, isopropanol, ethanol-acetate-ether 6:3:1, acetone-ethanol-ether 6:3:1, benzene, hexane, methanol-chloroform 1:1, methanol-chloroform-water 2:1:3 and chloroform-methanol 2:1 (Sweeney and Weihrauch, 1976). More recently, other solvents have been investigated. These include methanol-benzene 4:1 (Lepage and Roy, 1986), petroleum ether, ethyl ether (Lewis et al., 1991a) hot benzene and diethyl ether (Kaneda et al., 1980). Though differences in extraction efficiency among these solvents exist, it appears that chloroform-methanol and methanol-benzene are easily among the two best, and recovery studies using these two solvents indicate values of greater than 95% (Keneda et al., 1980; Lepage and Roy, 1986). Solvent mixtures which contain both polar and nonpolar compounds yield more complete cholesterol extraction from food products in which cholesterol may be bound to a lipoprotein or other substance in the food (Sweeney and Weihrauch, 1976).

Separation or Purification of Cholesterol

In the 1940's and 1950's many studies used the Liebermann-Burchard color producing reagent which was added directly to the solvent extract of the food. Data from these extracts tend to be higher in mg% cholesterol concentration than those in which more elaborate purification procedures were used (Kuo et al., 1992). Many interfering substances can cause these values to be
higher. They include phytosterols, keto steroids (progesterone, testosterone, estradiol, dehydroisoandrostenone, cholestenone) (Stadtman, 1957), bile acids, vitamin D, bilirubin, bromide, hemoglobin, iodide, bromate, iodate, nitrite, nitrate, vitamin A, pyramidone, sodium salicylate, thiouracil and polyunsaturated fatty acids may interfere with the color reaction (Lillienberg and Svanborg, 1976). Furthermore, LaCroix et al., (1973) showed that direct colorimetric analysis of chloroform-methanol extracts without isolating the cholesterol from impurities in the extract resulted in overestimated cholesterol content because of the solution turbidity. For these reasons, direct addition methods have not been used recently. Instead other colorimetric methods have been developed (Searcy and Berquist, 1960; Sperry and Webb, 1963; Parekh and Jung, 1970). Rhee et al. (1982a), used the procedure developed by Searcy and Berquist, (1960) and other investigators have used this method or made subtle modifications for saponification (Wheeler et al., 1987; Bohac et al., 1988; Swize et al., 1992). These improved colorimetric methods were almost as time consuming as the AOAC GC method reported by Kuo et al. (1992).

Cholesterol can also be isolated by precipitation. Three compounds used for precipitating cholesterol include digitonin, tomatine and saponin. Of these three, digitonin has been studied the most intensively. Digitonin precipitates free cholesterol, but not its esters. Cholesterol can then be split from its digitonide by treatment with benzene, xylene, dimethyl sulfoxide or pyridine. Therefore, to determine total cholesterol, the extract can be saponified to change cholesterol esters to free cholesterol. Mild saponification is preferred as Sperry and Brand (1943) and Bohac and Rhee (1988) found that strong saponification
followed by neutralization with HCl resulted in compounds producing extraneous colors and overestimated cholesterol content.

In addition to being isolated by precipitation, cholesterol can also be isolated using chromatographic techniques. These would encompass column, thin layer and gas-liquid procedures. Because free cholesterol is more strongly absorbed on the column than cholesterol esters, it elutes after cholesterol esters. Stepwise elution is accomplished with mixtures of polar and non-polar solvents. As a mixture becomes more polar free cholesterol is eluted. Common elution solvents include ethyl ether with petroleum ether and chloroform with petroleum ether (Sweeney and Weihrauch, 1976).

Organic solvents used for chromatograms include the following: cyclohexane-benzene 4:1, cyclohexane-heptane 1:1, ethyl ether-petroleum ether 1:1, benzene-methanol 9:1, hexane-ethyl ether-acetic acid 80:20:2, hexane-benzene 5:2, hexane-benzene 90:20, chloroform-methanol 2:1 and chloroform-methanol-water 65:25:4.

In separating cholesterol by gas liquid chromatography, various column coatings have been investigated. Sheppard et al. (1977) compared columns packed with 3% OV-1, 3% OV-17, 3% OV-225, mixture of 1% each of OV-1, OV-17 and OV-225, 3% JXR, 5% SE-30 and 1% SE-30. Of these, OV type columns did not satisfactorily resolve the campesterol and stigmasterol butyrates. The 1% SE-30 was selected over the 5% because elution time was more rapid and sensitivity was better. Otherwise, SE-30 columns were equally as useful for efficient separation using gas liquid chromatographic techniques.
Procedures to Measure Cholesterol

Most methods to measure cholesterol contents in food are those that originated for serum cholesterol and have undergone minor modification. Cholesterol assay methods can generally be divided into three groups, colorimetric, enzymatic and chromatographic. These three methods will be the major focus; however, two other methods, gravimetry and titration will be discussed briefly. The original methods of Windaus (1910) and Sperry and Webb (1963) are based upon cholesterol's reaction with digitonin and hence, gravimetric determination of precipitated cholesterol digitonide. This method has drawbacks. It is a relatively slow procedure that lacks sensitivity. Also, it is not totally specific for cholesterol and would include phospholipids present (Sweeney Weihrauch, 1976). Titration with I₂ or KI on unused K₂Cr₂O₇ cholesterol digitonide has been investigated by Okey (1930) and Haenni (1941) who found this method lacks specificity since compounds which can be oxidized would interfere with the reaction.

Colorimetric

The most widely used color reaction has been one involving the Liebermann-Burchard reagent. This reaction uses chloroform, acetic anhydride and sulfuric acid for color development. Numerous researchers (Hirsch and Ahrens 1958; Kelsey, 1939; Sperry and Brand, 1943; Sperry and Webb, 1950; Sperry and Webb, 1963) have used this method. Interfering substances react colorimetrically (Gardner and Williams, 1921), cholesterol esters react more rapidly than free cholesterol (Sperry and Brand, 1943), the reaction has a low
sensitivity, is time dependent, light labile and moisture affected (Crawford, 1958). Consequently, this method has undergone tremendous modification.

Several alternatives to the Liebermann-Burchard color reagent have been investigated. A color reagent consisting of acetic acid, acetic anhydride and sulfuric acid was first used by Schoenheimer and Sperry, (1934). It was subsequently revealed by Sperry and Webb (1953) that this procedure did not adequately remove excess digitonin. Consequently, interfering colors evolved which overestimated the cholesterol content.

Another such widely used colorimetric procedure was that of Zlatkis et al., (1953). They used a reagent consisting of FeCl₃, glacial acetic acid and sulfuric acid and added it to samples previously dissolved in acetic acid. Since free cholesterol and cholesterol esters produced the same color, saponification was not necessary. Interferences were detected, however, from bilirubin (Chiamori and Henry, 1959), unsaturated acids (Rhodes, 1959), and bromine (Manasterski and Zak, 1973). An improved reagent was developed by Parekh and Jung (1970) using uranium acetate, ferrous sulfate, glacial acetic acid, and sulfuric acid. This method was a modification of the Searcy and Berquist (1960) procedure. This method precipitated proteins and extracted cholesterol and overcame interference by other chromogens. This was an improvement over the Zlatkis et al. (1953) method which used FeCl₃ reagent and was found to incompletely remove interferences and was affected by temperature.

In general, colorimetric procedures are not totally specific. They seem to have interference from chemicals reacting unfortunately as the desired ones (Witte et al., 1974). Nevertheless, these methods are inexpensive, some use simple
extraction techniques, and depending on substance being analyzed, may yield results in close agreement with enzymatic or chromatographic procedures.

**Enzymatic Procedures**

Because of the clinical importance of testing for cholesterol and analytical difficulties associated with colorimetric methods, determination of cholesterol by enzymatic procedures has been intensively studied. Classically, colorimetric methodology has revolved around dehydration and dimerization reactions of cholesterol in the presence of metal catalysts and strong acids. Colorimetric reactions have long been plagued with difficulties of lack of specificity, sensitivity and standardization. Also, results have been technician dependent (Witte et al., 1974). In 1974, Allain and co-workers sought to develop an enzymatic method that would increase specificity and simplicity. This cholesterol procedure was the first attempt to incorporate the specificity of a totally enzymatic procedure into a single aqueous reagent. This procedure had good sensitivity, did not require cholesterol extraction from the organic phase, or sample dilution and avoided the use of harsh mineral acids. However, the reaction in the presence of cholesterol analogs (3-β OH compounds) all showed some positive error, although was less than Abell et al. (1953) method for sterols in normal serum. Even though absolute specificity was not achieved with this method, it was more specific than non-enzymatic procedures.

Enzymatic reactions and subtle modifications are as follows:
Cholesterol esters are first hydrolyzed by cholesterol esterase to produce cholesterol. Cholesterol is then oxidized by cholesterol oxidase to choleste-4-en-3-one and hydrogen peroxide. The hydrogen peroxide produced is then coupled with the chromogen, 4 aminoantipyrine, and p-hydroxybenzenesulfonate in the presence of peroxidase to yield a quinoneimine dye which has an absorbance max at 500 nm. Intensity of the color produced is directly proportional to total cholesterol in the sample. Interfering substances such as bilirubin; 3-β OH compounds and 7 dehydrocholesterol and 7-cholesten-3β-ol have been determined. (Sigma Diagnostics procedures No. 352)

**Gas Chromatography**

Because of their greater specificity, methods for determining cholesterol based on gas-liquid chromatography are usually more accurate than colorimetric or enzymatic procedures. This is especially true when samples being assayed contain interfering sterols along with other interfering
substances. In fact in 1976, the AOAC first acted upon this method of
determination of cholesterol in multicomponent foods and in 1977 final action
by AOAC was taken to adopt it. The principle of this method is that the lipid is
extracted by a mixed solvent and saponified. The unsaponifiable fraction
containing cholesterol and other sterols is extracted with benzene. Sterols are
derivatized to form trimethylsilyl ethers which are determined quantitatively
by gas chromatography using 5 α cholestane as an internal standard (AOAC,
1990).

Comparison of Methods

A recent study conducted by Kuo et al. (1992) compared a colorimetric
method with the AOAC GC method (AOAC, 1990) for 13 meat and dairy
products. They found no statistical difference for the colorimetric procedure for
10 of 13 foods analyzed although GC values were lower and had coefficient
variations range from 0.5% to 5.5%. Furthermore, the colorimetric method had
a detection limit of 10.8 mg/100 gm of food if the moisture content was 50% or
greater. The correlation coefficient between the two methods was 0.9992. The
authors mention that this method is applicable only to foods which cholesterol
is the only major sterol and significant amounts of phytosterols would interfere
with the measurement and give higher results. Ulberth and Reich (1992)
reported that although enzymatic determination of sterols is not strictly specific
for cholesterol, the content in foods of animal origin determined enzymatically
agreed favorably with chromatographically generated data for frankfurters and
sausage. However, when this enzymatic procedure was applied to foods
containing both animal and vegetable fats, the enzymatic procedure
overestimated true cholesterol content. This is because sterols with a 3-β OH
group, including phytosterols, react with the enzyme.

In 1976, Lillienberg and Svanborg compared GC with enzymatic and
colorimetric procedures for serum and plasma. They found no difference
between GC and enzymatic, although colorimetric values were significantly
higher by 12% than those obtained with GC. Nakagawa et al. (1977) concluded
in a comparison of two colorimetric procedures compared with GC and
enzymatic that the enzymatic procedure gave lower values than either of the
colorimetric methods, the GC procedure was the most suitable method for
determination of cholesterol content in foods containing several neutral sterols
because of its specificity and precision.

Factors Influencing Cholesterol Content of Beef

Degree of Marbling

The USDA quality grading system is designed to segregate beef on the
basis of its expected palatability. To accomplish this, a subjective appraisal of
marbling degree in the longissimus muscle between the twelfth and thirteenth
rib is made by a USDA grader. Historically, Choice is the quality grade of retail
expectation. USDA Select, formally known as Good, has gained substantially in
popularity by many consumers who prefer leaner beef. In addition, many high
quality, white-tablecloth restaurants use USDA Prime beef in their trade. More
consumers seem to be conscious of cholesterol content in foods they typically
consume. Yet, with typical fresh meat merchandising practices, little
information is available to them regarding cholesterol content of beef with different degrees of marbling.

A few studies have evaluated the influence that marbling has on cholesterol content of beef longissimus muscle. One of the first studies was conducted by Stromer et al. (1966). They reported no significant differences (P>.05) in cholesterol content among raw beef rib steak varying in marbling degree. Cholesterol values reported ranged from 36 to 46 mg/100 gm while the tissue marbling ranged from practically devoid to moderately abundant (Table 1). These researchers used the method of Schoenheimer and Sperry (1934) to determine cholesterol content. These values are lower than most others in the literature. This is due to the extraction methodology. This group extracted lipid from lyophilized muscle with chloroform only. This alone may not have efficiently or completely extracted the polar lipids of which muscle contains appreciable amounts.

<table>
<thead>
<tr>
<th>Marbling Score</th>
<th>Cholesterol Content (mg/100 gm) from beef longissimus muscle varying in degree of marbling and maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maturity A</td>
</tr>
<tr>
<td>Moderately abundant</td>
<td>45</td>
</tr>
<tr>
<td>Slightly abundant</td>
<td>43</td>
</tr>
<tr>
<td>Modest</td>
<td>43</td>
</tr>
<tr>
<td>Small</td>
<td>41</td>
</tr>
<tr>
<td>Traces</td>
<td>46</td>
</tr>
<tr>
<td>Practically devoid</td>
<td>46</td>
</tr>
</tbody>
</table>
Surprisingly, little information was reported until 1982 when Rhee et al. (1982a) published results from a study comparing degree of marbling and cookery on cholesterol content in beef strip loin steaks. Specifically, they compared beef steaks which ranged in marbling from practically devoid to moderately abundant and cooked these steaks to either 60°C or 75°C using an oven preheated to 177°C. Values ranged from 52–66 mg raw to 77-92 mg/100 gm cooked (Table 2). These results were obtained by using the cholesterol procedure of Searcy and Berquist (1960).

Table 2. Cholesterol content (mg/100 gm) of raw and cooked beef strip loin steaks varying in degree of marbling and internal endpoint temperature

<table>
<thead>
<tr>
<th>Marbling Score</th>
<th>Cholesterol Content</th>
<th>Internal Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>60°C</td>
</tr>
<tr>
<td>Practically devoid</td>
<td>51.8y</td>
<td>76.7</td>
</tr>
<tr>
<td>Small</td>
<td>64.0x</td>
<td>78.3</td>
</tr>
<tr>
<td>Slight</td>
<td>60.0x</td>
<td>81.3</td>
</tr>
<tr>
<td>Traces</td>
<td>60.1x</td>
<td>81.4</td>
</tr>
<tr>
<td>Modest</td>
<td>65.9x</td>
<td>80.2</td>
</tr>
<tr>
<td>Moderate</td>
<td>61.4x</td>
<td>87.1</td>
</tr>
<tr>
<td>Slightly abundant</td>
<td>62.5x</td>
<td>87.4</td>
</tr>
<tr>
<td>Moderately abundant</td>
<td>64.7x</td>
<td>86.4</td>
</tr>
</tbody>
</table>

x,y Means within the same column with different superscripts differ (p<.05).
For uncooked steaks, only those containing the practically devoid degree of marbling were significantly different (p<.05) when compared with those from other marbling classes. Steaks cooked to 75°C had a higher (p<.01) cholesterol content than those cooked to 60°C, however, there were no differences among marbling class within a particular degree of doneness. Therefore, differences observed for raw steaks containing a practically devoid degree of marbling were not apparent after cooking.

Berg, et al. (1985) compared rib steaks which varied in marbling from practically devoid to moderate which were cooked either by broiling or microwaving to approximately 70°C. They reported data on a dry matter basis only. This is not only inconsistent with the manner in which food is consumed, but also is difficult to compare their results to most other studies (Table 3). These data were determined using the cholesterol procedures of Stadtman (1957). More recently, Hoelscher et al. (1988) compared effects of quality grade, cooking, and subcutaneous fat trim level on cholesterol content

<table>
<thead>
<tr>
<th>Marbling Score</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>180\textsuperscript{a}</td>
</tr>
<tr>
<td>Modest</td>
<td>180\textsuperscript{a}</td>
</tr>
<tr>
<td>Small</td>
<td>197\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Slight</td>
<td>194\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Traces</td>
<td>184\textsuperscript{a}</td>
</tr>
<tr>
<td>Practically devoid</td>
<td>211\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} Means within the same column with different superscripts differ (p<.05).
of strip loin steaks. They found no difference (p>.05) in raw steaks ranging in quality grade from Select through Prime. A significant difference (p<.05), however, was found as cooked Prime steaks contained a higher content of cholesterol than cooked Choice or Select steaks (Table 4). Steaks were cooked using a Faberware Open-Hearth broiler to a 70°C degree of doneness. These data were determined according to the cholesterol procedure described by Rhee et al. (1982b) with slight modifications for saponification.

Table 4. Cholesterol content (mg/100 gm) of raw and cooked beef strip loin steaks which varied in quality grade

<table>
<thead>
<tr>
<th>Quality Grade</th>
<th>Prime</th>
<th>Choice</th>
<th>Select</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>63.5</td>
<td>62.0</td>
<td>60.9</td>
</tr>
<tr>
<td>Cooked</td>
<td>84.5a</td>
<td>78.8b</td>
<td>74.4b</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts differ (p<.05).

Findings of these studies would suggest minimal differences in cholesterol content of cooked steaks which vary widely in degree of marbling. Therefore, selecting leaner cuts of beef with expectations of reducing cholesterol intake may not actually give the desired result and meet consumer expectations.

Distribution of Cholesterol

Hoelscher et al. (1988) investigated subcellular distribution of cholesterol in muscle and adipose tissue. To accomplish this, a method for determination
of cholesterol in both membrane-bound and storage component fractions was developed and used on beef strip loin steaks that varied in quality grade, cooking, and subcutaneous fat trim level. After subjecting tissue to fractionation techniques nearly identical to Das et al. (1981) and lipid extraction using the modified Folch et al. (1957) procedure, cholesterol was determined according to the procedures of Rhee et al. (1982b) with modifications for saponification. They reported that between 18 to 36 percent of the cholesterol in raw steak is contributed by marbling (storage component form) and 22 to 36 percent for cooked steak, depending on quality grade. Furthermore, subcellular distribution of cholesterol in raw and cooked adipose tissue would indicate that nearly 90 and 80 percent, respectively, would be in the storage form (Table 5). The subcellular distribution in muscle tissue changed when intramuscular fat was increased. Therefore, as amount of non-membrane lipid increased (per gram), membrane material was decreased (per gram) causing a change in distribution. Cholesterol in the membrane component decreased while the lipid storage component of cholesterol increased, possibly due to the effect of increased lipid "diluting" the membranous component of muscle tissue fibers. This resulted in no overall change of cholesterol. This would likely explain why Prime muscle tissue had a higher subcellular fractional percentage of cholesterol (p<.05) in the storage component compared with Choice or Select simply because Prime has a higher percentage of intramuscular fat. After cooking, all three quality grade levels maintained the same subcellular distribution of cholesterol as was observed for raw samples.

Unlike the compensation observed in subcellular fractions of cholesterol in muscle tissue when intramuscular fat was increased, this did not occur in
Table 5. Cholesterol content (mg/100 gm) and subcellular fractionation of beef strip loin steak and subcutaneous adipose tissue

<table>
<thead>
<tr>
<th>Class</th>
<th>Raw</th>
<th>Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prime</td>
<td>63.5</td>
<td>64.0a</td>
</tr>
<tr>
<td>Choice</td>
<td>62.0</td>
<td>79.5b</td>
</tr>
<tr>
<td>Select</td>
<td>60.9</td>
<td>81.1b</td>
</tr>
<tr>
<td>Adipose Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prime</td>
<td>121.2</td>
<td>9.0</td>
</tr>
<tr>
<td>Choice</td>
<td>115.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Select</td>
<td>113.5</td>
<td>11.3</td>
</tr>
</tbody>
</table>

a,b Means within same column with different superscripts differ (p<.05)

adipose tissue, either raw or cooked. Cholesterol contributed by the adipose tissue membrane portion remained unchanged.

This study has provided greater understanding of the partitioning of cholesterol between muscle and adipose tissue. This data helped clarify results of previous findings—why total cholesterol (per gram) in raw muscle does not increase substantially as intramuscular fat increases, and why adipose tissue has considerably more cholesterol (per gram) than muscle tissue.

Regression Equations

Few investigators have determined correlations between fat and cholesterol content. In an early comparison which involved longissimus, semimembranosus, serratus ventralis, semitendinosus, and psoas major muscles, Tu et al. (1967) reported that cholesterol content increased slightly
with a rise in percent lipid. Indirectly, these authors concluded that marbling fat contained slightly more cholesterol than muscle. Rhee et al. (1982a) reported a correlation coefficient between lipid content and cholesterol content was highly significant \( p < .001 \) in beef longissimus muscle ranging in marbling from practically devoid to moderately abundant. They found correlation coefficients for lipid content and cholesterol amount were similar for both raw and cooked steaks (Table 6).

Furthermore, Lewis et al. (1991b) evaluated cholesterol content in the longissimus muscle of steers fed forage or grain diets based on differences in extracted lipid. For grain finished cattle, extracted fat \( p < .01 \) was related to cholesterol content. Surprisingly, carcass marbling had a higher correlation coefficient with cholesterol than extracted lipid (0.42 versus 0.35). For the forage fed group, a curvilinear relationship \( p < .05 \) between cholesterol and extracted lipid was observed. This was contrasted, however, to the linear relationship observed for grain fed cattle. The authors stated the reason for the curvilinear relationship observed in forage fed cattle were higher cholesterol contents at low and high fat concentrations (Table 6).

In a recent study reported by Khatri (1992), it was shown that the relationship between cholesterol and lipid content was highly significant \( p < .001 \) in longissimus muscle from bulls and steers.

In general, these equations generated by different investigations are very similar and show a relationship with increasing cholesterol content slightly by increased amounts of lipid.
Table 6. Correlation coefficients for extracted lipid and cholesterol content and prediction equations for cholesterol content based on lipid content

<table>
<thead>
<tr>
<th>Study</th>
<th>Correlation Coefficient</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tu et al. (1967)</td>
<td>none available</td>
<td>Y = 48.9 + 1.7 (Lipid Content)</td>
</tr>
<tr>
<td>Rhee et al. (1982a)</td>
<td>Cholesterol and lipid content</td>
<td>Y = 54.73 + 0.87 (Lipid Content)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R² = 0.39</td>
</tr>
<tr>
<td>Lewis et al. (1991b)</td>
<td>Grain Fed: Cholesterol and Lipid Content</td>
<td>Y = 43.71 + 0.81 (Lipid Content)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R² = 0.35</td>
</tr>
<tr>
<td>Forage Fed: Cholesterol and Lipid Content</td>
<td>Y = 55.03 + 0.99 (Lipid Content) + (Lipid Content)²</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R² = 0.16</td>
</tr>
<tr>
<td>Khatri (1992)</td>
<td>Cholesterol content and lipid content</td>
<td>Y = 44.1 + 1.83 (Lipid Content) bulls</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R² = 0.438</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y = 48.9 + 0.72 (Lipid Content) steers</td>
</tr>
</tbody>
</table>

Cooking Methods Comparison

Studies have been conducted which compare various cookery methodology to cholesterol content. Two studies have been conducted using ground beef that varied in initial fat content (Ono et al., 1985; Hoelscher et al., 1987). Broiling, roasting and grilling were compared by Ono et al., (1985) on ground beef patties that had an initial fat content of 18.4, 21.5 or 27.0 percent. They found only one significant difference. Low fat formulated patties roasted had a higher cholesterol content than low fat patties broiled or grilled. No other differences were reported. Values reported ranged from 70.7 to 85.3 mg/100 gm (raw) to 97.7 to 110.1 mg/100 gm (cooked) and were generated using gas chromatographic techniques (Table 7).
Furthermore, Hoelscher and co-workers (1987) evaluated broiling versus pan frying ground beef patties that initially had 1.2, 4.7, 10.0, 14.8, 20.2, 26.3 and 31.1 percent fat. No difference in cholesterol content of cooked patties was observed for patties regardless of preparation method or initial fat content. Cholesterol contents were graphically depicted on a per patty basis (113.4 gm). Converting their data to a 100 gm basis would provide cholesterol concentrations in cooked ground beef from 63 to 70 mg (Table 8). These data were generated using the colorimetric cholesterol procedure of Searcy and Berquist (1960) as described by Rhee et al. (1982a) with modifications of Bohac et al. (1988). Prusa and Hughes (1986) investigated how cholesterol content in pork tenderloin muscle was influenced by cooking in three oven types. They were conventional, convection and microwave. Conventional and convection ovens were preheated to 163°C and degree of doneness was closely monitored. Degree of doneness for microwave prepared samples was monitored by a microwave thermometer placed in the center of each cut. No significant differences were observed for cholesterol content of pork tenderloin cuts prepared in the various ovens. However, samples prepared by microwaving were numerically higher. Cholesterol contents reported varied from 67.1 to 70.7 wet weight basis 156.3 vs 176.3 mg/100 gm on a dry matter basis (Table 9). These data were determined using the method of Searcy and Berquist (1960) as described by Rhee et al. (1982a) with slight modifications for saponification. Morgan et al., (1988) reported that cholesterol content of pork top loin chops braised, pan fried or roasted were similar whereas, those chops that were microwaved had a slightly higher cholesterol content which ranged from 79.8 to 88.9 mg/100 gm (Table 10). These data were determined using the method of Searcy and Berquist (1960) as described by Rhee et al. (1982a), with slight
modifications for saponification. Berg et al. (1985) compared broiling and microwave cookery and found broiled rib steaks had significantly less cholesterol than those cooked in a microwave. They used the colorimetric method of Stadtman (1957) to determine cholesterol. It is difficult to compare their results with most previously published reports since they expressed their data on a dry matter basis instead of the more common "as is" basis and graphically depicted fat, protein and dry matter. Nevertheless, actual values reported ranged from 145 to 255 mg/100 gm on a dry matter basis. In addition, Wheeler et al. (1987) reported that beef longissimus muscle broiled to 70°C had a higher cholesterol content (27 percent) than raw samples (Table 11).

In general, with the exception of microwave cookery, it appears that traditional cookery methods such as broiling, roasting and frying have little influence on cholesterol content of cooked product. Microwave cookery, however, perhaps tends to concentrate the cholesterol in the final product by increasing evaporative losses.

Table 7. Effect of cookery method on cholesterol content (mg/100 gm) of ground beef differing in fat content

<table>
<thead>
<tr>
<th>Cholesterol Content</th>
<th>Cookery Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Fat Content</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>18.4</td>
<td>70.7</td>
</tr>
<tr>
<td>21.5</td>
<td>76.7</td>
</tr>
<tr>
<td>27.0</td>
<td>85.3</td>
</tr>
</tbody>
</table>

\(^a\) Broiled using Faberware Open-Hearth broiler.  
\(^b\) Roasting by convection oven at 192°C.  
\(^c\) Faberware electric griddle temperature set at 177°C.
Table 8. Effect of cookery method on cholesterol content (mg/100 gm) of ground beef differing in fat content

<table>
<thead>
<tr>
<th>Fat Content</th>
<th>Cookery Method</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulated</td>
<td>Actual</td>
<td>Raw</td>
</tr>
<tr>
<td>0</td>
<td>1.2</td>
<td>68.8</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>67.9</td>
</tr>
<tr>
<td>10</td>
<td>10.0</td>
<td>67.0</td>
</tr>
<tr>
<td>15</td>
<td>14.8</td>
<td>66.1</td>
</tr>
<tr>
<td>20</td>
<td>20.2</td>
<td>70.5</td>
</tr>
<tr>
<td>25</td>
<td>26.3</td>
<td>68.8</td>
</tr>
<tr>
<td>30</td>
<td>31.1</td>
<td>79.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Broiled using Faberware Open-Hearth broiler.

<sup>b</sup> Electric skillet temperature of 149°C.

Table 9. Cholesterol content (mg/100 gm) of pork tenderloin steak cooked by different methods

<table>
<thead>
<tr>
<th>Cookery Method</th>
<th>Wet weight basis</th>
<th>Dry matter basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formed</td>
<td>Conventional&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Convection&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wet weight basis</td>
<td>67.2&lt;sup&gt;x&lt;/sup&gt;</td>
<td>65.7&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry matter basis</td>
<td>158.6&lt;sup&gt;x&lt;/sup&gt;</td>
<td>156.3&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Oven temperature was 163°C.

<sup>b</sup> Power setting 368 W.

<sup>x,y</sup> Means with different superscripts within a row differ (p<.05).

Table 10. Cholesterol content (mg/100 gm) of pork top loin chop cooked by different methods

<table>
<thead>
<tr>
<th>Cookery Method</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braising&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pan-Frying&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>85.6</td>
<td>81.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Browned at 117°C and heated in 163°C oven to 71°C.

<sup>b</sup> Heated in frying pan at 190°C to 71°C.

<sup>c</sup> Heated in 179°C oven to 71°C.

<sup>d</sup> Cooked at 50% of full power setting.
Table 11. Cholesterol content (mg/100 gm) of raw versus broiled beef longissimus muscle

<table>
<thead>
<tr>
<th>Status</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>63.3</td>
</tr>
<tr>
<td>Broiled</td>
<td>80.3</td>
</tr>
</tbody>
</table>

* Broiled in Faberware Open Hearth broiler.

**Effect of Degree of Doneness Comparisons**

Rhee et al. (1982a) evaluated effect of degrees of doneness on cholesterol content of longissimus muscle from strip loin steaks that varied widely in degree of marbling (practically devoid-moderately abundant). They cooked steaks from a frozen state (0°C) in a preheated oven at 177°C to an internal temperature of either 60°C or 75°C. They found a 22-48% higher cholesterol content in steaks cooked to 60°C and a 38-65% higher content in those cooked to 75°C compared with raw when expressed on a wet weight "as is" basis (Table 12). Furthermore, cholesterol values ranged from 51.8 to 65.9 mg/100 gm (raw) to 76.7 to 87.4 mg/100 gm (60°C) and 85.6 to 92.2 (75°C). When expressed on a dry weight basis, however, there was little difference in cholesterol content between cooked and uncooked steaks. Actual values ranged from 192.1 to 226.1 mg/100 gm (raw) and 186.1 to 254.3 mg/100 gm (cooked). These researchers used the colorimetric procedure of Searcy and Berquist (1960) to determine cholesterol content.

Prusa and Hughes (1986) compared cooking pork tenderloin steaks by conventional or convection oven preheated to 163°C with microwave cookery to two degrees of doneness. They investigated the effect of cooking to either 71°C or 77°C on cholesterol content (Table 13). They reported tenderloin chops cooked to 71°C had less cholesterol (60.4 mg/100 gm) than cooking to 77°C (75.2
mg/100 gm). Furthermore, when evaluated on a dry weight basis, tenderloin chops cooked to 71°C had less cholesterol (154.1 mg/100 gm) than those heated to 77°C (173.4 mg/100 gm.), whereas, raw values were 45.1 mg/100 gm (wet-weight basis) and 152.1 (dry-weight basis). The colorimetric method of Searcy and Berquist (1960) as described by Rhee et al., (1982a) was used to determine cholesterol contents.

Kregel et al. (1986) evaluated cholesterol content of broiled ground beef patties cooked to either 71°C or 77°C internal temperature. Patties were formulated to contain three levels of fat. These levels were 8.7, 20.4 and 27.8 percent (raw) and had a corresponding cholesterol content of 51.3, 58.2, and 59.8 mg/100 gm. After broiling, across all fat levels, patties cooked to 71°C had less cholesterol (68.8 mg/100 gm) than those cooked to 77°C (72.8 mg/100 gm) (Table 14). When expressed on dry weight basis, significant differences were not found for patties broiled to 71°C compared with 77°C (153.6 versus 154.7 mg/100 gm). The colorimetric procedures of Searcy and Berquist (1960) as described by Rhee et al. (1982a) were used to determine cholesterol.

Heating to a higher internal endpoint temperature increased or concentrated the cholesterol in tissues on a wet-weight basis. As expected, the moisture of cooked meat was inversely related to evaporative and total cook losses. Negative associations between cholesterol and moisture would substantiate the concentration of cholesterol in tissues occurs via evaporative losses due to cooking.

Cook Drip

Few investigations have evaluated cholesterol content present in cook drip. One such study conducted by Tu et al. (1967) reported that ground chuck
Table 12. Cholesterol content (mg/100 gm) of raw and cooked beef longissimus muscle from strip loin steaks with different degrees of marbling

<table>
<thead>
<tr>
<th>Marbling Degree</th>
<th>Raw Wet Wt.</th>
<th>Raw Dry Wt.</th>
<th>Cooked Wet Wt. 60°C</th>
<th>Cooked Wet Wt. 75°C</th>
<th>Cooked Dry Wt. 60°C</th>
<th>Cooked Dry Wt. 75°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>60°C</td>
<td>75°C</td>
<td>60°C</td>
<td>75°C</td>
</tr>
<tr>
<td>Moderately abundant</td>
<td>64.7Y</td>
<td>199.6</td>
<td>86.4</td>
<td>89.6</td>
<td>186.1</td>
<td>204.0Y</td>
</tr>
<tr>
<td>Slightly abundant</td>
<td>62.5Y</td>
<td>201.0</td>
<td>87.4</td>
<td>88.3</td>
<td>191.4</td>
<td>207.5Y</td>
</tr>
<tr>
<td>Moderate</td>
<td>61.4Y</td>
<td>192.0</td>
<td>87.1</td>
<td>90.0</td>
<td>195.7</td>
<td>201.5Y</td>
</tr>
<tr>
<td>Modest</td>
<td>65.9Y</td>
<td>226.1</td>
<td>80.2</td>
<td>92.2</td>
<td>193.7</td>
<td>210.3Y</td>
</tr>
<tr>
<td>Small</td>
<td>64.0Y</td>
<td>213.5</td>
<td>78.3</td>
<td>90.8</td>
<td>213.5</td>
<td>194.9XY</td>
</tr>
<tr>
<td>Slight</td>
<td>60.0Y</td>
<td>229.0</td>
<td>81.4</td>
<td>85.7</td>
<td>204.5</td>
<td>254.3X</td>
</tr>
<tr>
<td>Traces</td>
<td>60.1Y</td>
<td>229.0</td>
<td>81.4</td>
<td>85.7</td>
<td>204.5</td>
<td>254.3X</td>
</tr>
<tr>
<td>Practically devoid</td>
<td>51.8X</td>
<td>212.7</td>
<td>76.7</td>
<td>85.6</td>
<td>218.4</td>
<td>235.5X</td>
</tr>
</tbody>
</table>

b Steaks cooked from frozen state (0°C) to either 60°C or 75°C in oven preheated to 177°C.

x,y Mean values within a column with different superscripts differ (p<.05).

Table 13. Cholesterol content (mg/100 gm) of pork tenderloin steak heated to two different degrees of doneness

<table>
<thead>
<tr>
<th>Tissue Preparation</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degree of Doneness</td>
</tr>
<tr>
<td></td>
<td>71°C</td>
</tr>
<tr>
<td>Wet Weight</td>
<td>60.4b</td>
</tr>
<tr>
<td>Dry Weight</td>
<td>154.1X</td>
</tr>
</tbody>
</table>

a Compilation of data from conventional oven, convection oven and microwave oven prepared samples.

b,c Means within a row with different superscripts differ (p<.001).

x,y Means within a row with different superscripts differ (p<.01).
Table 14. Cholesterol content (mg/100 gm) of ground beef patties broiled to two different degrees of doneness\(^a\)

<table>
<thead>
<tr>
<th>Tissue Preparation</th>
<th>Cholesterol Content</th>
<th>Degree of Doneness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Weight</td>
<td>71°C</td>
<td>77°C</td>
</tr>
<tr>
<td>Dry Weight</td>
<td>153.6</td>
<td>154.7</td>
</tr>
<tr>
<td></td>
<td>68.8(^b)</td>
<td>72.8(^c)</td>
</tr>
</tbody>
</table>

\(^a\) Samples broiled in 180°C oven.
\(^b,c\) Means within a row with different superscripts differ (p<.05).

which had an initial fat content of either 15.6 or 29.7 percent fat raw, contained about 7 and 15 percent of the total cholesterol in the cook drip after broiling, respectively. These data were determined using the colorimetric cholesterol method described by Mann (1961).

Furthermore, Rhee et al. (1982b) determined cholesterol content in cook drip from strip loin steaks varying in marbling degree which were broiled to either 60°C or 75°C. The cholesterol content was higher in drip (p<.05) for steaks cooked to 75°C as opposed to 60°C. They found cook drip contained a small percentage of the cholesterol that was present in steaks initially (Table 15). These data were reported using the cholesterol method of Searcy and Berquist (1960).

Kregel et al. (1986) determined cholesterol content in cook drip from ground beef formulated to contain different fat levels. Patties were cooked to either 71°C or 77°C internal endpoint temperature. They found as percent fat in the formulation increased, a significantly higher percent (p<.01) cholesterol in drip compared with the total raw muscle (Table 16). Furthermore, they reported that as the fat content of the raw patties increased, the percentage of cholesterol retained by cooked beef patties decreased (p<.01) (Table 16).
Table 15. Cholesterol content in drippings (across all marbling groups) from steaks cooked to 60° or 75°C

<table>
<thead>
<tr>
<th>Marbling Score</th>
<th>60°C</th>
<th>75°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderately abundant</td>
<td>1.69</td>
<td>2.55</td>
</tr>
<tr>
<td>Slightly abundant</td>
<td>1.53</td>
<td>2.05</td>
</tr>
<tr>
<td>Moderate</td>
<td>2.20</td>
<td>2.60</td>
</tr>
<tr>
<td>Modest</td>
<td>0.82</td>
<td>1.46</td>
</tr>
<tr>
<td>Small</td>
<td>1.14</td>
<td>1.35</td>
</tr>
<tr>
<td>Slight</td>
<td>0.90</td>
<td>0.94</td>
</tr>
<tr>
<td>Traces</td>
<td>0.29</td>
<td>2.30</td>
</tr>
<tr>
<td>Practically void</td>
<td>0.69</td>
<td>0.60</td>
</tr>
</tbody>
</table>

When evaluating differences related to endpoint temperature, they reported that the percent of cholesterol in drip compared to raw muscle, it was found that the amount increased (p<.01) only for ground beef containing 9.5 percent fat when it was heated to a higher internal temperature. This relationship did not exist for ground beef at higher fat levels (Table 17).

Table 16. Cholesterol content relationships in cook drip from ground beef patties cooked compared with raw

<table>
<thead>
<tr>
<th>Fat Percentage</th>
<th>9.5</th>
<th>21.0</th>
<th>28.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol in drip as compared to total raw muscle percentage</td>
<td>0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol retention percentage</td>
<td>96.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means within same row with different superscripts differ (p<.05).
Data show steaks and patties which contained higher amounts of fat, and consequently, higher amounts of cholesterol, had a proportionately higher amount of cholesterol present in the cook drip. This tended to negate differences in cholesterol content which could be related to fat content of the raw product. Also, not surprising is the finding that cooking to a higher internal temperature tended to increase cholesterol content of cook drip.

Table 17. Interaction between percent fat and internal endpoint temperature for percent cholesterol in drip as compared to total raw muscle of cooked ground beef patties

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Fat,%</th>
<th>71°C</th>
<th>77°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drip-Cholesterol, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.5</td>
<td>1.5^a</td>
<td>4.5^b</td>
<td></td>
</tr>
<tr>
<td>21.0</td>
<td>6.3^c</td>
<td>6.5^c</td>
<td></td>
</tr>
<tr>
<td>28.5</td>
<td>6.8^c</td>
<td>6.3^c</td>
<td></td>
</tr>
</tbody>
</table>

a,b Means which are not followed by a common letter are significantly different (p<0.01).

Cholesterol Content of Adipose Tissue

Several studies, reviewed chronologically, have been conducted to determine cholesterol quantities in adipose tissue and data appears in Table 18. Del Vecchio et al. (1955) found that adipose tissue trimmed from round and rib steaks contained 82 mg of cholesterol per 100 gm of tissue cholesterol. Stromer and co-workers (1966) investigated effects of carcass maturity and subcutaneous layer (internal or external) on cholesterol content of subcutaneous adipose tissue over the ribeye between the eleventh and thirteenth rib. Carcasses chosen were assumed to represent approximate animal ages corresponding to:
A, 15-18 months; B, 20-24 months; and F, over 6 years. They found the external subcutaneous fat layer had cholesterol contents of 132 mg/100 gm, 150 mg/100 gm, and 138 mg/100 gm for A, B, and F, respectively, where A maturity was lower (p<.05) than B maturity. For the internal layer, they reported 113 mg/100 gm for A, B and F maturity groups, respectively, where F was higher (p<.05) than A or B. These investigators used the cholesterol procedure of Schoenheimer and Sperry (1934) to quantify cholesterol.

Tu et al. (1967) reported that beef fat from the ventral side (plate/flank) had a cholesterol content of 56.2 mg/100 gm whereas beef fat from the dorsal region had 67.8 mg/100 gm. These data were generated using a modification of procedures used by Mann (1961).

Rhee et al. (1982b) investigated changes in cholesterol content of subcutaneous fat from ribeye steaks and intermuscular fat before and after cooking. They determined that raw subcutaneous fat had a cholesterol content of 114.3 mg/100 gm compared with 124.1 mg/100 gm for cooked. Furthermore, they determined raw intermuscular (seam) fat contained 108.2 mg/100 gm and this increased to 110.3 mg/100 gm after cooking. In addition, this group dissected a sample of intramuscular fat from five ribeye steaks using a microscope. They reported marbling fat contained a cholesterol content of 104.6 mg/100 gm. This compares favorably with Kinney Sweeten et al. (1990) who found cholesterol averaged 118 mg/100 gm of intramuscular adipose tissue with 54 percent in the cytoplasm and 46 percent in the membranes. These data were determined using procedures described by Rhee et al. (1982a) with slight modifications for saponification.
Eichhorn et al. (1986a) determined cholesterol content from adipose tissue from bulls and steers removed at two different anatomical locations, over the twelfth rib and perenephric fat. Gender effects were not statistically different. However, subcutaneous fat contained 101.7 mg/100 gm whereas perenephric fat contained 89.7 mg/100 gm. These investigators used the procedure of Leffler (1959) to assess the quantity of cholesterol and incorporated the saponification procedures used by Rhee et al. (1982b). In an unrelated study, Eichhorn et al. (1986b) evaluated the same adipose tissue locations in 7 to 10 year old cows that were subjected to one of three dietary treatments. They reported that subcutaneous fat from mature cows had a cholesterol content of 151.8 mg/100 gm (initial), 139.7 mg/100 gm (maintenance), and 87.1 mg/100 gm (ad libitum). In addition, they determined that perenephric fat from these cows had a cholesterol content of 128.1 mg/100 gm, and 100.3 mg/100 gm for maintenance and ad libitum, respectively. The investigators explanation for the cholesterol content being lowest in adipose tissue from mature cows on the high energy diet may be in large part due to increased amount of triacylglycerol deposited in the fat and hence, diluting the cholesterol concentration.

Wheeler et al. (1987) determined cholesterol content of adipose tissue from the beef round in a study which compared two cattle types (British and Chianina), two sex types (steer and heifer), and four time on feed intervals (0, 77, 128, and 182 days). Of these variables, none were statistically different. Therefore, they reported that subcutaneous round fat contained 98.9 mg/100 gm using the cholesterol procedures of Rhee et al. (1982a). Hoelscher and coworkers (1988) determined cholesterol content on subcutaneous fat from strip loin steaks which were trimmed to either 0.64 cm or 1.27 cm (subcutaneous fat)
and then broiled. They reported raw subcutaneous fat had 115.0 mg/100 gm and 118.9 mg/100 gm (raw) or 95.5 mg/100 gm and 110.2 mg/100 gm (cooked) for 0.64 cm and 1.27 cm trim levels, respectively. The finding of having a lower value for cooked adipose tissue than raw was consistent with Agricultural Handbook 8-13 (USDA 1986) (99 mg/100 gm raw and 95 mg/100 gm cooked). However, this conflicts with data reported by Rhee et al. (1982b) which showed cooked adipose tissue had a higher cholesterol content than raw adipose tissue. In part, this may be partially explained by the cooking procedures used in that study as steaks were cooked from a frozen state rather than the thawed.

Table 18. Cholesterol content (mg/100 gm) of raw and cooked adipose tissue from various locations

<table>
<thead>
<tr>
<th>Cholesterol Content</th>
<th>Adipose Tissue Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del Vecchio et al. (1955)</td>
<td>82.0 from round and rib steaks</td>
</tr>
<tr>
<td>Stromer et al (1966)</td>
<td>132.0 external layer (11th-13th rib steak)</td>
</tr>
<tr>
<td>Tu et al. (1967)</td>
<td>113.0 internal layer (11th-13th rib steak)</td>
</tr>
<tr>
<td>Rhee et al. (1982b)</td>
<td>56.2 side</td>
</tr>
<tr>
<td>Eichhorn et al. (1986a)</td>
<td>67.8 dorsal</td>
</tr>
<tr>
<td>Eichhorn et al. (1986b)</td>
<td>114.3 rib steak</td>
</tr>
<tr>
<td>Tu et al. (1967)</td>
<td>101.7 12th rib (bulls and steers)</td>
</tr>
<tr>
<td>Wheeler et al. (1987)</td>
<td>87.1-151.8 12th rib-averaged on 7-10 year old cows on dietary study</td>
</tr>
<tr>
<td>Hoelscher et al. (1988)</td>
<td>98.9 round</td>
</tr>
<tr>
<td>Hoelscher et al. (1988)</td>
<td>117.0 strip loin</td>
</tr>
</tbody>
</table>

Table 18. Cholesterol content (mg/100 gm) of raw and cooked adipose tissue from various locations

<table>
<thead>
<tr>
<th>Raw Subcutaneous</th>
<th>Cooked Subcutaneous Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoelscher et al. (1988)</td>
<td>117.0 strip loin</td>
</tr>
<tr>
<td>Rhee et al. (1982b)</td>
<td>114.3 rib steak</td>
</tr>
</tbody>
</table>

Table 18. Cholesterol content (mg/100 gm) of raw and cooked adipose tissue from various locations

<table>
<thead>
<tr>
<th>Raw vs. Cooked Seam Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoelscher et al. (1988)</td>
</tr>
<tr>
<td>Rhee et al. (1982b)</td>
</tr>
</tbody>
</table>

Table 18. Cholesterol content (mg/100 gm) of raw and cooked adipose tissue from various locations

<table>
<thead>
<tr>
<th>Raw Marbling Fat Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhee et al. (1982b)</td>
</tr>
<tr>
<td>Kinney Sweeten et al. (1990)</td>
</tr>
</tbody>
</table>
Effect of Subcutaneous Fat Trim Level

To assess whether cholesterol can migrate from subcutaneous fat into the lean upon cooking, a few studies have been conducted to investigate cholesterol migration. Hoelscher et al. (1988) evaluated the effect of trimming subcutaneous fat on strip loin steaks from USDA Prime, Choice and Select strip loin steaks to 1.27 cm, 0.64 cm or completely removing this fat prior to cooking. Steaks were prepared on Faberware Open-Hearth broilers to an internal temperature of 70°C. They found no difference in longissimus muscle cholesterol content in steaks regardless of the trimmable subcutaneous fat remaining. The values ranged from 60.7 to 64.7 mg/100 gm (raw) and 77.2 to 80.8 mg/100 gm (cooked). The raw subcutaneous adipose tissue contained between 115.0 to 118.9 mg/100 gm, whereas cooked adipose tissue from steaks having 1.27 cm had 110.2 versus 95.5 mg/100 gm for steaks possessing 0.64 cm subcutaneous fat (Table 19). These data were determined using the colorimetric cholesterol procedures of Searcy and Berquist (1960) as described by Rhee et al. (1982a) with slight modifications of Bohac et al. (1988).

Berg et al. (1985) also investigated effects of cookery (broiling versus microwaving) on cholesterol content of beef rib steaks which varied in marbling from practically devoid to moderate that either had the subcutaneous fat removed or remaining (0.6cm). Steaks with fat removed before cooking had less cholesterol in the longissimus muscle than those which had fat on during cooking, although, this increase was not consistent across all marbling classes. On a dry matter basis, values ranged from 171 (fat removed) to 209 (fat on) mg/100 gm using the colorimetric cholesterol procedure of Stadtman (1957). Morgan et al. (1988) found no difference for cholesterol content of pork top loin
chops regardless of whether the chops had 1.27 cm or the subcutaneous fat was completely removed (Table 20). These chops were cooked by braising, pan-frying, roasting and microwaving. Data were determined by using the cholesterol method of Searcy and Berquist (1960) as described by Rhee et al. (1982a) with modifications of Bohac et al. (1988).

In general, it appears cholesterol migration from subcutaneous fat has not been shown to occur in beef strip steaks trimmed to 0, 0.64 cm or 1.27 cm of subcutaneous fat in a paired comparison analysis (Hoelscher et al., 1988). Furthermore Morgan et al. (1988) in a paired cut experimental design using pork top loin chops found no difference in cholesterol content for those chops having subcutaneous fat trimmed completely or 1.27 cm. However, Berg et al. (1985) showed that steaks with 0.6 cm of subcutaneous fat before cooking had more cholesterol than those that were trimmed free of fat.

Therefore, it seems somewhat inconclusive to completely rule out cholesterol migration into the lean until more definitive studies are conducted.

Table 19. Cholesterol content (mg/100 gm) of raw and cooked beef longissimus muscle and subcutaneous fat from strip loin steak that varied in trim level

<table>
<thead>
<tr>
<th>Class</th>
<th>Cholesterol Content</th>
<th>0.0 cm</th>
<th>0.64 cm</th>
<th>1.27 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw longissimus muscle</td>
<td></td>
<td>60.7</td>
<td>61.0</td>
<td>64.7</td>
</tr>
<tr>
<td>Cooked longissimus muscle</td>
<td></td>
<td>80.8</td>
<td>77.2</td>
<td>79.7</td>
</tr>
<tr>
<td>Raw subcutaneous fat</td>
<td></td>
<td>115.0</td>
<td>118.9</td>
<td></td>
</tr>
<tr>
<td>Cooked subcutaneous fat</td>
<td></td>
<td>95.5b</td>
<td>110.2c</td>
<td></td>
</tr>
</tbody>
</table>

* Steaks were cooked on Faberware Open Hearth broiler to a 70°C internal endpoint.
* Means within the same row with different superscripts differ (p<.05).
Table 20. Cholesterol content (mg/100 gm) of cooked pork, top loin chop, knife-separable lean as affected by trim level.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trim Level</td>
</tr>
<tr>
<td>0.0 cm</td>
<td>83.2</td>
</tr>
<tr>
<td>1.27 cm</td>
<td>84.8</td>
</tr>
</tbody>
</table>

*Data compiled from samples cooked by braising, pan-frying, roasting, and microwaving.*

Ground Beef

With consumer preferences toward lowering consuming less fat, calories and cholesterol, lean ground beef alternatives are becoming popular. However, scientific data do not support the assumption that selecting ground beef lower in fat will reduce fat and cholesterol consumption.

Tu et al. (1967) broiled ground chuck patties varying in fat content. They found that slight differences existed in the broiled edible portion and cook drip (Table 21). These data were generated using the cholesterol procedure of Mann (1961). Little information was provided as to the number of observations within each of these sample groups. Furthermore, it would have been desirable to report data on an "as is" wet weight tissue basis for cooked patties.

Rhee and Smith (1983) investigated effect of cooking on cholesterol content of patties containing different amounts of beef, textured soy protein and

Table 21. Percentage of fat and cholesterol content(mg/100 gm) of raw and broiled ground chuck patties varying in initial fat content

<table>
<thead>
<tr>
<th>Ground Chuck</th>
<th>Raw Fat(%)</th>
<th>Raw Edible Portion</th>
<th>Drip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>15.6</td>
<td>72.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Sample 2</td>
<td>29.7</td>
<td>74.6</td>
<td>12.5</td>
</tr>
</tbody>
</table>
fat. These investigators used a 3 x 4 factorial design where three levels of fat (8.4, 16.2, and 27.0) and four levels of textured soy protein (TSP) (0, 10, 20, and 30 percent) were used in the formation of the patties. They observed that as fat content increased in raw patties and TSP decreased, cholesterol content was lower (P<.05) (Table 22). These patties were cooked to 75°C by heating in an oven preheated to 177°C. There was a significant interaction between TSP and fat level (P<.001) (Table 23).

Table 22. Cholesterol content of raw beef patties

<table>
<thead>
<tr>
<th>Fat %</th>
<th>Rehydrated TSP Content of Raw Beef Patties</th>
<th>Means For Effect of Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg cholesterol / 100 gm</td>
<td></td>
</tr>
<tr>
<td>8.4</td>
<td>60.6 55.6 46.8 40.7</td>
<td>50.9b</td>
</tr>
<tr>
<td>16.2</td>
<td>62.9 55.3 48.4 45.3</td>
<td>53.0b</td>
</tr>
<tr>
<td>27.0</td>
<td>63.3 57.6 53.7 48.4</td>
<td>55.8a</td>
</tr>
<tr>
<td>Means for effect of TSP</td>
<td>62.3a 56.2b 49.6c 44.8d</td>
<td>52.3a 49.6b 48.4c 45.3d</td>
</tr>
</tbody>
</table>

a,b,c,d Means within a column or row with different superscripts differ (P<.05).

At initial fat levels of 8 and 16 percent, the amount of cholesterol in 100 gm of cooked sample tended to decrease as the amount of TSP increased, however, the decreases in cholesterol content were not proportional to the increase in the amount of TSP as was the case in the initial cooked patties. At an initial fat level of 27 percent, amount of cholesterol per cooked patty did not decrease with an increase in the amount of TSP. Interestingly, of patties containing no TSP, those which had an initial fat level of 27 percent had a lower cholesterol content after cooking than those which had 8 or 16 percent. Though not determined in this study, it was speculated these patties lost a substantial
Table 23. Cholesterol (mg/100 gm) content of cooked beef patties

<table>
<thead>
<tr>
<th>Fat(%)</th>
<th>Rehydrated Textured Soy Protein (%)</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>8.4</td>
<td>91.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>16.2</td>
<td>86.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>27.0</td>
<td>74.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means in the same row with different superscripts differ (p<.05). Means in the same column sideline are not different (p>.05).

amount of cholesterol in the drip during cooking. Cholesterol data in this study were determined using the cholesterol assay procedure of Searcy and Berquist (1960).

In a study which compared extra lean, lean, and regular ground beef, Ono et al. (1985) evaluated effects of cookery by broiling (Farberware Open Hearth broiler), roasting (convection oven set at 192°C) and grilling (Farberware Electric Griddle set at 177°C) on cholesterol content. They found a significant difference (p<.05) in raw ground beef as the extra lean treatment had a lower cholesterol content than regular. No differences (p>.05) were observed after cooking, regardless of heating method or fat level (Table 24). These values appear to be higher than data from most previously published reports, although the trends are certainly similar. This investigation used a modified AOAC gas chromatography procedure to determine cholesterol content.

Kregel et al. (1985) determined cholesterol content by evaluating three fat levels, two internal endpoint temperatures, and two storage periods for ground beef patties. These patties were cooked in an oven preheated to 180°C to either 71°C or 77°C internal endpoint temperature. Samples, both raw and cooked,
Table 24. Cholesterol content (mg/100 gm) of raw and cooked ground beef patties differing in fat content and cookery method

<table>
<thead>
<tr>
<th>Fat (%)</th>
<th>Cooking Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
</tr>
<tr>
<td>18.4</td>
<td>70.7&lt;sup&gt;c&lt;/sup&gt;y</td>
</tr>
<tr>
<td>21.5</td>
<td>76.7&lt;sup&gt;bxy&lt;/sup&gt;</td>
</tr>
<tr>
<td>27.0</td>
<td>85.3&lt;sup&gt;b&lt;/sup&gt;x</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means in the same row with different superscripts differ (P<.05).
<sup>x,y</sup> Means in the same column with different superscripts differ (P<.05).

were analyzed at day 0 and after 30 days of storage at -20°C. Raw, low fat patties had less cholesterol (p<.05) than the intermediate or high fat raw samples (Table 25). No differences in cholesterol content, however, were attributable to variation in initial fat content after the patties were cooked (Table 25). Patties had less cholesterol after 30 days frozen storage, regardless of whether they were raw or cooked (Table 26).

Table 25. Cholesterol content (mg/100 gm) of raw and cooked ground beef patties differing in fat content

<table>
<thead>
<tr>
<th>Fat, %</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.5</td>
</tr>
<tr>
<td>Raw</td>
<td>51.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooked</td>
<td>71.6</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row with different superscripts differ (P<.05).
Table 26. Effect of storage on cholesterol content of ground beef.

<table>
<thead>
<tr>
<th>Storage Time, Days</th>
<th>Status</th>
<th>0</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>59.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cooked</td>
<td>75.9&lt;sup&gt;x&lt;/sup&gt;</td>
<td>65.7&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row with different superscripts differ (P<.005).

<sup>x,y</sup> Means in the same row with different superscripts differ (P<.01).

Explanation of observed reduction in cholesterol values after 30 days of storage could possibly be related to cholesterol breakdown and oxidation. Little is known about conditions which may cause cholesterol oxidation. Yet this is an area which clearly should be more thoroughly investigated since cholesterol oxidation products may have a very detrimental role in the formation of atherosclerotic lesions. Furthermore, patties cooked to 77°C had a higher cholesterol content (p<.05) than those heated to 71°C (72.8 mg/100 gm versus 68.8 mg/100 gm, respectively). These data were determined using the method of Searcy and Berquist (1960) as described by Rhee et al. (1982a) with slight modifications for saponification.

Hoelscher et al. (1987) compared cholesterol content in ground beef patties that were formulated to contain various fat levels and cooked either by broiling or pan-frying and then frozen. A subsample was then reheated by microwave oven and refrozen. They used the procedures described by Rhee et al. (1982a) with slight modifications for saponification to determine cholesterol content. These patties had initial fat levels of 1-2, 5, 10, 15, 20, 25, and 30 percent. They found cholesterol content of ground beef patties was not related
to \( p < .05 \) fat content, either raw or cooked. There was a significant increase in those raw patties formulated to contain 30 percent fat. The data was depicted graphically, however cholesterol content of the raw ground was approximately 68 mg/100 gm. Cholesterol values (per patty basis) of cooked patties tended to be lower than raw patties due to losses during cooking. Although, when expressed as a percentage, cooked patty values were higher due to the loss of sample weight during cooking. Furthermore, microwave reheating of cooked patties yielded no significant change in cholesterol content of broiled or pan-fried patties.

These studies reveal that although differences may exist for raw ground beef varying in initial fat content where samples containing higher amounts of fat generally have a higher amount of cholesterol. After cooking these differences are diminished. Therefore, consumers wishing to purchase lean ground beef with the thought of reducing cholesterol intake, will likely not accomplish this objective.

**Effect of Storage Length**

Hood and Allen (1971) investigated the chemical stability of cholesterol in rib steak which had been postmortem stored either 2, 7, 14, or 21 days at 2°C. They found no difference \( p > .05 \) in cholesterol content in the longissimus muscle, regardless of postmortem storage length (Table 27). These data were generated by the cholesterol procedure of Leffler (1963). Kregel et al. (1986) evaluated raw and cooked ground beef patties which varied in fat content from 9.5 to 28.5 percent fat that had been stored frozen (-20°C) after forming (raw) or cooking. They observed raw patties stored 30 days had less cholesterol \( p < .05 \)
Table 27. Effects of postmortem storage length on cholesterol content (mg/100gm) of beef rib steak (lean-only)

<table>
<thead>
<tr>
<th>Postmortem Storage Time (days)</th>
<th>2</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Content</td>
<td>52</td>
<td>54</td>
<td>49</td>
<td>50</td>
</tr>
</tbody>
</table>

compared with those evaluated at time of formulation. Furthermore, cooked patties stored for 30 days had less cholesterol ($p<.001$) when compared with those analyzed immediately after cooking (Table 28). These data were generated using the cholesterol method described by Rhee et al. (1982a) with slight modifications for saponification. Cholesterol may oxidize during storage of cooked muscle foods to which antioxidants are not added. This is because conditions present in both cooking and cold storage may potentiate autoxidation of cholesterol and contribute to its breakdown. Data reported by Kregel et al. (1986) seems to support this. Much of the ground beef utilized by the institutional trade is precooked and frozen stored to be later reheated. Research efforts should be undertaken to more thoroughly investigate conditions necessary and underlying mechanisms which may potentiate autoxidation of cholesterol and the implication to human health.

Cattle Diets

Few beef studies have been conducted which compare diet to tissue cholesterol content. Eichhorn et al. (1986b) evaluated different dietary energy levels fed to mature cows (7 to 10 years of age) and the influence on cholesterol content of certain lean tissues and fat. In muscle, no differences could be
attributed to dietary-energy level for samples from the longissimus or triceps brachii. Longissimus muscle cholesterol content ranged from 54.3 mg/100gm to 56.9 mg/100gm while triceps brachii cholesterol content varied from 60.5 mg/100gm to 62.8 mg/100 gm (Table 29). However, dietary energy levels influenced adipose tissue cholesterol to a large extent. Subcutaneous fat from the twelfth rib and pereneprhic fat showed a similar relationship in that as dietary energy level increased, cholesterol content in the adipose tissue decreased. These values ranged from 87.1 mg/100 gm to 151.8 mg/100 gm for subcutaneous fat and 100.3 mg/100 gm to 128.1 mg/100 gm for pereneprhic fat for high energy to low energy rations, respectively (Table 29). The authors speculate that additional triacylglycerol in adipose tissue of cows fed high energy diets could have diluted the concentration of cholesterol in the tissue enough to create lower values for cholesterol content. Additional evidence, including data on cell membrane content is needed for clarification of mechanisms involved.

In addition, Lewis et al. (1991a) evaluated the influence of feeding 24 steers either a concentrate grain diet for approximately 210 days or a forage
Table 29. Cholesterol content (mg/100 gm) of muscle and adipose tissue from mature cows according to sampling site and feeding group

<table>
<thead>
<tr>
<th>Feeding Group</th>
<th>Sampling Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol Content</td>
</tr>
<tr>
<td></td>
<td>Longissimus Muscle</td>
</tr>
<tr>
<td></td>
<td>Triceps Brachii Muscle</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous Fat</td>
</tr>
<tr>
<td></td>
<td>Perenephric Fat</td>
</tr>
<tr>
<td>Initial (Low)</td>
<td>56.9</td>
</tr>
<tr>
<td></td>
<td>61.5</td>
</tr>
<tr>
<td></td>
<td>151.8</td>
</tr>
<tr>
<td></td>
<td>128.1</td>
</tr>
<tr>
<td>Maintenance</td>
<td>54.2</td>
</tr>
<tr>
<td></td>
<td>60.5</td>
</tr>
<tr>
<td></td>
<td>139.7</td>
</tr>
<tr>
<td></td>
<td>123.2</td>
</tr>
<tr>
<td>Ad Llibitum (high)</td>
<td>54.3</td>
</tr>
<tr>
<td></td>
<td>62.8</td>
</tr>
<tr>
<td></td>
<td>87.1</td>
</tr>
<tr>
<td></td>
<td>100.3</td>
</tr>
</tbody>
</table>

Ration for 330 days had on cholesterol content of the longissimus muscle. They found that forage fed steers had a cholesterol content of 43.4 mg/100 gm compared with 42.8 mg/100 mg for those fed a concentrate diet. In a similarly designed study with 120 steers, they reported forage fed steers had a cholesterol content of 47.6 mg/100 gm in longissimus muscle while those fed the concentrate diet had a content of 47.3 mg/100 gm (Lewis et al. 1991b). These data are about 10 mg/100 gm lower than most values for raw longissimus muscle. Unlike many investigators that can trace cholesterol methodology back to Searcy and Berquist (1960) via Rhee et al. (1982a) or Mann (1961), these researchers utilized more modern and specific laboratory instrumentation by determining cholesterol quantity with HPLC techniques (Brown, 1987).

In general, these studies would suggest that dietary energy level has little influence on tissue cholesterol concentration. This is not surprising considering how ruminant species metabolize nutrients and that cholesterol is distributed in a subcellular sense, in both membrane and storage components. Cholesterol content in muscle than those fed tallow and lard.
Jacobson et al. (1974) fed calves reconstituted milks containing 3.5% lard, tallow, or refined soybean oil. They reported soybean oil fed calves had higher

Weyant et al. (1976) observed no effect of diet treatment on cholesterol content of cow muscle when fed safflower oil which was protected from the rumen hydrogenation by a protein-formaldehyde coating.

Garrett et al. (1976) found no influence of animal fat on cholesterol content of the meat and fat for steers fed protected polyunsaturated vegetable oils or tallow.

Bohac and Rhee, (1988) evaluated whether animal diet modification would affect cholesterol content of beef muscles at three anatomical locations. These sites were the longissimus, semimembranosus and semitendinosis. They formulated diets to contain either 0% or 20% canola rapeseed. No differences were reported for anatomical locations or dietary treatment. They observed steers fed 20% canola rapeseed had a cholesterol content 55.4 mg/100 gm while those fed 0% had 58.7 mg/100 gm. These data were determined using Rhee et al. (1982a) cholesterol procedures with modifications for the saponification step to include an antioxidant, propyl gallate (Bohac et al., 1988).

**Time on Feed**

Of the few studies which have evaluated effects of time on feed and cholesterol content of tissue, experimental evidence would suggest this variable has little influence on cholesterol content. Wheeler et al. (1987) evaluated raw and cooked longissimus muscle, and subcutaneous fat of the round from carcasses of animals fed 0, 77, 128, and 182 days. Cholesterol content of raw or cooked longissimus muscle and subcutaneous fat were not responsive (p>.05) to
differences in length of feeding (Table 30). Data were generated using the cholesterol procedure of Rhee et al. (1982a) using a slight modification for the saponification step.

Khatri, (1992) compared cholesterol content of longissimus muscle from bulls and steers that had been serially slaughtered at 180, 210, or 240 days after the feeding trial had been initiated. There were no significant differences reported for time on feed in that study, and the author did not report mean values. These data were generated using GCMS techniques.

Table 30. Cholesterol content (mg/100 gm) in various tissues from cattle differing in number of days on feed

<table>
<thead>
<tr>
<th>Cholesterol Content</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days on Feed</td>
<td>Raw Longissimus</td>
</tr>
<tr>
<td>0</td>
<td>62.4</td>
</tr>
<tr>
<td>77</td>
<td>64.3</td>
</tr>
<tr>
<td>128</td>
<td>63.2</td>
</tr>
<tr>
<td>182</td>
<td>63.4</td>
</tr>
</tbody>
</table>

It appears on the basis of these investigations that time on feed is one variable that has little influence over cholesterol content of the lean tissue and yielded a small, but non-significant effect on the content in fat tissue. Therefore, altering time on feed to reduce the amount of tissue cholesterol does not appear to be a feasible production alternative.
Effect of Yield Grade

Rhee et al. (1982b) compared cholesterol content of raw and cooked boneless rib steaks removed from the anterior end (6th rib) of each wholesale rib that ranged in USDA yield grade from 1 through 5. They found no significant difference (p<.05) within raw steaks or cooked steaks between different yield grades when evaluated on a whole steak basis (Table 31). These data were determined using the cholesterol method described by Rhee et al. (1982a). Depending on yield grade, 43 to 60 percent of the cholesterol present in uncooked, boneless rib steaks was found to originate from the separable fat (intermuscular and subcutaneous); the corresponding value for cooked steaks was 37 to 56 percent. For uncooked as well as cooked steaks, percentage of cholesterol contributed by separable fat increased significantly with numerical increase in yield grade.

Clearly, meat merchandising practices in recent years have been to trim subcutaneous fat to 0.25 cm or less and eliminate large deposits of

<table>
<thead>
<tr>
<th>Yield Grade</th>
<th>Cholesterol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
</tr>
<tr>
<td>1</td>
<td>78.3</td>
</tr>
<tr>
<td>2</td>
<td>78.9</td>
</tr>
<tr>
<td>3</td>
<td>79.4</td>
</tr>
<tr>
<td>4</td>
<td>83.6</td>
</tr>
<tr>
<td>5</td>
<td>85.2</td>
</tr>
<tr>
<td>Overall</td>
<td>81.1</td>
</tr>
</tbody>
</table>
intermuscular fat. Therefore, comparisons of whole muscle cuts varying widely in subcutaneous and intermuscular fat may have less relevance to today's consumers than before, simply because they are not available at the point of purchase.

Browning et al. (1990) recently compared cholesterol content of ten muscles excised from each of eight carcasses selected for leanness (average yield grade 1.7) and those of typical carcasses (average yield grade of 3.0). They found a significant difference (p<.05) in cholesterol content of raw tissue for the lean versus typical carcass composites. Values were 67.1 versus 68.9 mg/100gm, respectively for lean and typical. Furthermore, after broiling, no differences in cholesterol content of lean or typical carcass composites were detected and values were 90.1 mg/100 gm, respectively. Furthermore, the investigators found that supraspinatus and infraspinatus muscle had the highest content of cholesterol on a raw basis (75-76 mg/100 gm), whereas the semitendinosus and longissimus dorsi had the smallest content (59-64 mg/100 gm, respectively). After broiling, longissimus muscle had 77 mg/100 gm and the infraspinatus contained the most (104.1 mg/100 gm). These data were determined according to procedures described by Rhee et al. (1982a) from Searcy and Berquist (1960) with modifications for saponification reported by Wheeler et al. (1987).

Effects of Animal/Carcass Age

Studies have been conducted to evaluate the effects that animal/carcass age has on the quantity of cholesterol present in tissues. Indirectly, Lewis et al. (1991b) evaluated a fast growing and slow growing developmental production systems (pasture feeding versus feedlot finishing). They found cholesterol
concentration was related to age in pasture developed steers (20 months of age) while it was not in feedlot developed steers (14 months of age). The difference, though statistically significant, may be of little practical importance when considering the cholesterol difference for fast and slow maturing animals in that the pasture fed treatment was 2.8 mg/100 gm higher (45.3 versus 48.1 mg/100 gm). This difference, in large part, could be due to the sensitivity of the HPLC techniques (Brown, 1987) that these investigators used to quantify cholesterol and the small standard error associated with each mean.

Wheeler et al. (1987) slaughtered cattle that ranged initially from 9-12 months of age to 15 to 18 months (182 day feedlot finishing period) of age for the final slaughter. They reported no difference in longissimus muscle cholesterol content of either of these groups. On a raw basis, 62.4 versus 63.4 mg/100 gm and on a cooked basis 78.6 and 81.6 mg/100 gm were observed for longissimus muscle for youngest and oldest, respectively. Round fat contained 92.3 mg/100 gm (youngest) or 98.9 mg/100 gm (oldest) and may be related to the moisture:lipid ratio which decreases as maturity advances. They used the cholesterol procedures of Rhee et al. (1982a).

Stromer et al., (1966) compared cholesterol content of longissimus muscle removed from carcasses of A, B and F (what is presently considered D to E) maturing beef carcasses. They found cholesterol content of longissimus muscle did not change with maturity. The methods used for analyzing cholesterol were adapted from Schoenheimer and Sperry (1934). Eichhorn et al., (1986a) determined cholesterol content of various tissues from 7 to 10 year old cows. They found cholesterol content in longissimus muscle contained 54.9 mg/100 gm, triceps brachii 61.5 mg/100 gm, subcutaneous fat 124.5 mg/100 gm.
and perenephric fat 115.0 mg/100 gm. These data were determined using the cholesterol procedures as described by Eichhorn et al. (1986b) and Leffler (1959). Although only a few studies have been conducted, it seems that animal age has little to do with the cholesterol content of the meat.

**Gender Differences**

Wheeler et al., (1987) evaluated strip loin steaks from steers and heifers to determine if sex class was influential in the amount of cholesterol contained in the tissue. They found no differences related to sex class. Raw longissimus muscle contained 63.2 and 63.5 mg/100gm for steers and heifers, respectively. On a cooked basis this same muscle had 80.7 and 79.9 mg/100gm, whereas the subcutaneous round fat contained 98.4 and 99.4 mg/100gm, respectively for steers and heifers (Table 32). Wheeler et al., (1987) used the procedure described by Rhee et al., (1982a) to determine cholesterol content.

Eichhorn et al., (1986) evaluated bulls and steers to determine if cholesterol content differences existed in muscle or adipose tissue. They found no difference related to sex class. Across sampling sites (longissimus, triceps brachii, semitendinosus, perenephric fat and subcutaneous fat), bulls had an average tissue cholesterol content of 75.4 mg/100 gm while steers had 75.7 mg/100 gm (Table 32). These investigators used the method of Leffler (1959) for cholesterol analysis and followed procedures by Rhee et al., (1982) for saponification of sample in the isolation steps.

Terrell et al., (1969) evaluated tissue from steers and heifers carcasses from various anatomical regions to determine if gender significantly influenced tissue cholesterol content. They found no differences statistically
attributed to sex class. When averaged across weight classes (386 kg, 420 kg and 455 kg) and various anatomical locations (psoas major, longissimus, transverse abdominis, semimembranosus, triceps brachii and semitendinosus), steer tissue had 79.9 whereas heifer tissue contained 75.7 mg/100 gm (Table 32). These data were determined using the procedures of Mann (1961).

Hood and Allen (1971) compared cholesterol content of longissimus muscle from half-sib bulls, steers, and heifers. They found heifers had a higher cholesterol content (p<.05) when compared with bulls and steers. They reported that bull, steer, and heifer longissimus muscle contained 46 mg/100 gm, 49 mg/100 gm, and 57 mg/100 gm of cholesterol, respectively (Table 32). These investigators used the procedures of Leffler (1963) to quantify cholesterol.

In a recent study, Khatri (1992) determined cholesterol content of beef longissimus muscle from bulls and steers subjected to three different time on feed intervals. In that study, cholesterol content of bull longissimus muscle was 51.6 mg/100 gm compared with 52.9 mg/100 gm for their castrate counterparts. There was a significant sex x lipid content interaction, although in all cases, cholesterol content means for steers and bulls within a lipid content grouping did not vary by more than 1.4 mg/100 gm (Table 32). These data were determined using a gas chromatography mass spectrometry procedure which traces its origin to Lepage and Roy (1986).

In general, the literature supports the consensus that no apparent difference in cholesterol content of beef muscle can be attributed to gender.
Influence of Breed Type

Few studies have evaluated cholesterol content from tissues of cattle that differed in breed type. Eichhorn et al. (1986b) compared cholesterol content from longissimus muscle, triceps brachii, perenephric fat, and twelfth rib fat from mature cows representing fifteen different breeds and crosses. No differences (p>.05) attributable to breed type were determined. They used the procedure of Leffler (1959) and a slight modification of the saponification procedure (Table 33).

In addition, Wheeler et al. (1987) compared two breed types and the influence it had on cholesterol content of raw and cooked longissimus muscle and raw subcutaneous round fat. These investigators observed no differences (p>.05) in content based on breed type (Table 33). This data was generated using

Table 32. Cholesterol content (mg/100 gm)differences for beef muscle(s) or fat based on gender differences

<table>
<thead>
<tr>
<th></th>
<th>Bull</th>
<th>Steer</th>
<th>Heifer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terrell et al. (1969)b</td>
<td>—</td>
<td>79.9</td>
<td>79.7</td>
</tr>
<tr>
<td>Hood and Allen (1971)e</td>
<td>46b</td>
<td>49b</td>
<td>57c</td>
</tr>
<tr>
<td>Eichhorn et al. (1986a)f</td>
<td>75.4</td>
<td>75.7</td>
<td>—</td>
</tr>
<tr>
<td>Wheeler et al. (1987)e</td>
<td>—</td>
<td>63.2</td>
<td>63.5</td>
</tr>
<tr>
<td>Khatri, (1992)e</td>
<td>51.6</td>
<td>52.9</td>
<td>—</td>
</tr>
</tbody>
</table>

b,c Mean in same row with different superscripts differ (p<.05).
d Data obtained from raw psoas major, Longissimus, transverse abdominis, semimembranosus, Triceps brachii, and semitendinosus.
e Data obtained on raw longissimus muscle.
f Data obtained from raw longissimus muscle, Triceps brachii, Semitendinosis, Perenephric adipose, and subcutaneous adipose.
the cholesterol method of Rhee et al. (1982a) with a slight modification of the 
saponification step.

Koch et al. (1987) compared the cholesterol content in lean tissue from 
Hereford and Brahman cattle. These breeds were found to contain similar 
amounts of cholesterol (p>.05) (Table 33).

Rouse and Beitz, (1988) reported that differences were not significant 
(P>.05) for small, medium, and large framed steers originating from the Rhodes 
Research Farm animal breeding project. The small framed cattle were 
composed of the following breeds; 1/4 Angus, 1/4 Jersey, and 1/2 initial female 
(hereford x Angus). The medium framed cattle consisted of 1/8 Jersey, 1/8 
Simmental, 1/4 Angus, and 1/2 initial female. And the large framed cattle 
were 1/4 Angus, 1/4 Simmental, and 1/2 initial female (Table 33). This group 
used an Auto-Analyzer technique and the values they report are much higher 
than most data generated by other researchers.

Lewis et al. (1991b) evaluated the influence that four growth types of 
cattle had on cholesterol content of the longissimus muscle. These cattle had 
been placed on typical high concentrate feedlot diets or on a forage based diet. 
Growth types were characterized by the authors as large size-slow maturing and 
were represented by the Chianina, Charolais, and crosses with these breeds; 
intermediate size-slow maturing type by the Red Poll and Hereford breeds; 
intermediate size-fast maturing type by the modern Angus breed; and small 
size-fast type by the small framed Angus cattle such as was popular in the 
United States back in the 1950's. Within or between feeding or breed type class, 
the range for cholesterol content differed by less than 3 mg/100mg. The 
absolute values these authors report are lower than most reported in the
Table 33. Effect of breed types on cholesterol content of tissue

<table>
<thead>
<tr>
<th>Eichhorn et al. (1986b) Breed-Type</th>
<th>Tissue</th>
<th>Result for Breed Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereford</td>
<td>Longissimus</td>
<td>all (p &gt; .05)</td>
</tr>
<tr>
<td>Angus</td>
<td>Triceps brachii</td>
<td>all (p &gt; .05)</td>
</tr>
<tr>
<td>Hereford x Angus</td>
<td>Pericranial fat</td>
<td>all (p &gt; .05)</td>
</tr>
<tr>
<td>Angus x Hereford</td>
<td>Subcutaneous fat</td>
<td>all (p &gt; .05)</td>
</tr>
<tr>
<td>Red Poll x Angus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Poll x Hereford</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown Swiss x Angus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown Swiss x Hereford</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelbvieh x Angus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelbvieh x Hereford</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chianina x Angus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chianina x Hereford</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maine Anjou x Angus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maine Anjou x Hereford</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Longissimus Raw</th>
<th>Longissimus Cooked</th>
<th>Subcutaneous Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheeler et al. (1987) Chianina</td>
<td>62.7</td>
<td>79.5</td>
</tr>
<tr>
<td>British Crossbred</td>
<td>63.9</td>
<td>81.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Koch et al. (1987) Breed</th>
<th>Longissimus Raw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereford</td>
<td>65.6</td>
</tr>
<tr>
<td>Brahman</td>
<td>64.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rouse and Beitz (1988) Frame Size</th>
<th>Longissimus Raw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Framed</td>
<td>(p &gt; .05)</td>
</tr>
<tr>
<td>Medium Framed</td>
<td>(p &gt; .05)</td>
</tr>
<tr>
<td>Large Framed</td>
<td>(p &gt; .05)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Averaged for Frame</th>
<th>Mean Content 106.9</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Frame Size-Maturity Type</th>
<th>Feedlot</th>
<th>Forage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis et al. (1991b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large Slow</td>
<td>45.6</td>
<td>45.3</td>
</tr>
<tr>
<td>Intermediate Slow</td>
<td>47.4</td>
<td>47.7</td>
</tr>
<tr>
<td>Longissimus Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate Fast</td>
<td>48.2</td>
<td>48.0</td>
</tr>
<tr>
<td>Small-Fast</td>
<td>48.3</td>
<td>48.1</td>
</tr>
</tbody>
</table>
literature and are likely a result of the specificity of the HPLC technique used (Brown, 1987).

Based on results from these previous investigations, few differences in cholesterol content of tissues can be traced back to breed type. This is not to imply that research approaches of combining genetic evaluation parameters to selection of lean beef cuts lower in cholesterol should be abandoned. However, the likelihood of success in finding a breed or breed type low in tissue cholesterol may be quite low since cholesterol is a biologically essential compound that functions in membrane fluidity.

General Background on Lipids

There is considerable concern and confusion among consumers, researchers, medical professionals, and government personnel about the role and fate of fat in meat, how preparation influences this, and concerns relative to appropriate levels of consumption. Lipids are defined as any group of substances which, in general, are soluble in ether, chloroform, or other solvents for fats, but are sparingly soluble in water (Dugan, 1976).

The following is a classification as reported by Dugan (1976) and contains the following elements useful in distinguishing many lipid substances.

General Classification of Lipids

A classification of lipids proposed by (Dugan 1976) contains the following elements which are useful in distinguishing the many lipid substances:

1. Simple lipids (neutral lipids)—esters of fatty acids with alcohols.
a. **Fats**: esters of fatty acids with glycerol

b. **Waxes**: esters of fatty acids with alcohols other than glycerol

2. **Compound lipids**—Compounds containing other groups in addition to ester or a fatty acid with an alcohol.
   a. **Phospholipids (phosphatides)**: esters containing fatty acids, phosphoric acid, and other groups usually containing nitrogen
   b. **Cerebrosides (glycolipids)**: compounds containing fatty acids, a carbohydrate and a nitrogen moiety, but no phosphoric acid
   c. **Other compound lipids**: sphingolipids and sulfolipids

3. **Derived lipids**—Substances derived from neutral lipids or compound lipids and having general properties of lipids.
   a. **Fatty acids**
   b. **Alcohols**: usually normal chain higher alcohols and sterols
   c. **Hydrocarbons**

**Fatty Acids**

Fatty acids are long chained hydrocarbons with a carboxyl group at one end and a methyl group on the other. They are unbranched and usually have an even number of carbon atoms. However, very low amounts of odd-numbered carbon chains of fatty acids may contain only a single bond between adjacent carbons. These are termed saturated fatty acids. In other carbon chains, one or more double bonds appear between adjacent carbon atoms, because they are not fully saturated with hydrogen, they are called unsaturated fatty acids. Those fatty acids with one double bond are called monounsaturated fatty acids and those with two or more are termed polyunsaturated fatty acids.
Common fatty acids in food fats are provided (Table 34) (CAST, 1991). The presence of a double bond in the fatty acid allows the molecule to maintain two different configurations, either cis or trans. In cis form the molecule is folded on itself at the double bond. In trans form, the fatty acid molecule is extended fully to its maximal length at the double bond (Figure 1). Unsaturated fatty acids in foods are predominately those of cis configuration, whereas those from trans configuration occur in small amounts from ruminants, yet are present in much higher amounts in vegetable oils which have been subjected to hydrogenation (CAST, 1991).

De novo synthesis of fatty acids in animals terminate with palmitic acid. Subsequently, this 16 carbon fatty acid may be elongated by a separate metabolic pathway. In addition, desaturase enzymes desaturate fatty acids at several positions in the chain. Animals, however, cannot desaturate beyond the Δ⁹ position of the carbon chain, unlike plants which have the ability to desaturate at positions Δ¹² and Δ¹⁵. This is why animals have a dietary requirement for linoleic and linolenic acids. Enzyme complexes occur in animal cells that desaturate at Δ⁵ if there is a double bond at the Δ⁹ position. Their enzymes are different from the Δ⁹ desaturase. Major polyunsaturated fatty acids are either derived from the diet or from desaturation and elongation of 18:2Δ⁹,12, 18:3 Δ⁹,12,15, or 20:4 Δ⁵,8,11,14. The elongation of C₁₆ and C₁₈ fatty acids to yield the C₂₀ to C₂₄ acids occurs by enzymatic addition of two carbon units. Synthesis of arachidonic acid (C₂₀:₄) from linoleic acid (C₁₈:₂) illustrates the principle by which polyunsaturated fatty acids are made in animals (CAST, 1991).

Fatty acid moieties in foods are not present in the free form. Instead, these are combined by ester linkage to a three carbon molecule of glycerol to
Table 34. Common fatty acids in food fats

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Common Name</th>
<th>Structure</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saturated Fatty Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>Butyric acid</td>
<td>CH₃(CH₂)₂COOH</td>
<td>-5.3</td>
</tr>
<tr>
<td>C6</td>
<td>Caproic acid</td>
<td>CH₃(CH₂)₄COOH</td>
<td>-3.2</td>
</tr>
<tr>
<td>C8</td>
<td>Caprylic acid</td>
<td>CH₃(CH₂)₆COOH</td>
<td>16.5</td>
</tr>
<tr>
<td>C10</td>
<td>Capric acid</td>
<td>CH₃(CH₂)₈COOH</td>
<td>31.6</td>
</tr>
<tr>
<td>C12</td>
<td>Lauric acid</td>
<td>CH₃(CH₂)₁₀COOH</td>
<td>44.8</td>
</tr>
<tr>
<td>C14</td>
<td>Myristic acid</td>
<td>CH₃(CH₂)₁₂COOH</td>
<td>54.4</td>
</tr>
<tr>
<td>C16</td>
<td>Palmitic acid</td>
<td>CH₃(CH₂)₁₄COOH</td>
<td>62.9</td>
</tr>
<tr>
<td>C18</td>
<td>Stearic acid</td>
<td>CH₃(CH₂)₁₆COOH</td>
<td>70.1</td>
</tr>
<tr>
<td></td>
<td>Unsaturated Fatty Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1</td>
<td>Palmitoleic acid</td>
<td>CH₃(CH₂)⁵CH=CH(CH₂)⁷COOH</td>
<td>0</td>
</tr>
<tr>
<td>C18:1</td>
<td>Oleic acid</td>
<td>CH₃(CH₂)⁷CH=CH(CH₂)⁷COOH</td>
<td>16.3</td>
</tr>
<tr>
<td>C18:2</td>
<td>Linoleic acid</td>
<td>CH₃(CH₂)⁴(CH=CHCH₂)²(CH₂)⁶COOH</td>
<td>-5</td>
</tr>
<tr>
<td>C18:3</td>
<td>Linolenic acid</td>
<td>CH₃CH₂(CH=CHCH₂)³(CH₂)⁶COOH</td>
<td>-11</td>
</tr>
<tr>
<td>C20:4</td>
<td>Arachidonic acid</td>
<td>CH₃(CH₂)⁴(CH=CHCH₂)⁴(CH₂)²COOH</td>
<td>-49.5</td>
</tr>
</tbody>
</table>

form triglycerides (Figure 2). Each fatty acid is combined by ester linkage to one of the alcohol groups of glycerol. Therefore, glycerol may be esterified with one, two, or three fatty acids, giving rise to mono, di, or triglycerides, respectively. Triglycerides are the predominant form of all lipids associated with food fats and are the primary storage form. Over 98 percent of the fatty acids in meats are in the form of triglycerides. Nearly all remaining fatty acids also are esterified components of the phospholipids, waxes, or cholesterol. Triglycerides may
contain several combinations of the fatty acids. If all three are the same, it is called a simple triglyceride. However, if two or more triglycerides differ, it is referred to as a mixed triglyceride, which predominate in food fats (CAST, 1991).

Figure 1. Geometric isomers of C₁₈:₁ fatty acid. Oleic acid is the cis form; elaidic acid is the trans form.

Figure 2. Structure of a generalized triacylglycerol. R₁, R₂, and R₃ represent three fatty acids.
Glycerophospholipids are the major type of phospholipids found in food fats. These contain two fatty acids esterified with two of the alcohol groups of the glycerol molecule (Figure 3).

The third alcohol group of glycerol is esterified with phosphoric acid which is esterified with another alcohol such as ethanolamine, choline, inositol, serine, glycerol, or phosphatidylglycerol. Glycerolphospholipids are major lipid components of cellular membranes. In animals the highest concentration is found in muscles and the lowest in adipose tissue. As the deposition of marbling fat increases, the contribution of fatty acids from glycerolphospholipids to total fatty acids or lipids decreases.

Glycerolphospholipids usually contain a higher proportion of unsaturated fatty acids than triglycerides in the same animals. Furthermore, these usually contain a saturated C₁₆ or C₁₈ fatty acid at the C-1 position of the glycerol and an unsaturated C₁₆ to C₂₀ fatty acid in the C-2 position. A higher proportion of polyunsaturated fatty acids incorporated in phospholipids as compared with triglycerides reflects the functional differences of these lipids. Whereas the
triglycerides are the primary storage form of lipids in the adipose tissue of animals, the phospholipids are structural components of cell membranes which function to allow the passages of molecules through them (CAST, 1991).

**Lipids in Muscle Structure**

The phospholipid and cholesterol portions of muscle lipids are essential because of their regulatory roles in the structure and function of the muscle cell and its organelles. Neutral lipids are not essential to muscle, however, they provide fatty acids for energy which are involved in normal metabolism in living muscle tissue and contribute certain characteristics to meat such as flavor, color stability, texture, juiciness, protein stability, shelf-life, and caloric content. Cholesterol and phospholipid fractions are generally considered to be membrane associated. However, the neutral lipids are present as droplets within muscle cells, fat cells or located within the perimysial connective tissue of muscle (Moody and Cassens, 1968). Those droplets within muscle are known as marbling.

**Fatty Acid Composition**

Fatty acid composition of muscle has distinct differences among different species. These differences represent one of the most significant variables in determining the palatability, processing, and storage characteristics of different muscle foods (Allen and Foegeding, 1981).

Fatty acids in ruminant tissues are the most saturated because there is normally extensive hydrogenation by microorganisms in the rumen (McDonald et al., 1977). These microorganisms can influence lipid metabolism
by a couple of different mechanisms. These are by the synthesis of microbial lipids or by changes in the dietary lipid prior to being digested in the intestine. Hydrogen gas which is present as a by-product of microbial fermentation serves as a source of hydrogen and is used by microbes for hydrogenation of C18 unsaturated fatty acids consumed by ruminants (Dryden et al., 1973; McDonald et al., 1977).

Because of hydrogenation in the rumen, fatty acid composition of the depot fat of mature cattle has been reported fairly consistently by a large number of investigators and that diet does have some influence. (Hornstein et al., 1961; Waldman et al., 1965; O'Keefe et al., 1968; Waldman et al., 1968; Terrell et al., 1969; Dryden and Marchello, 1970; Link et al., 1970; Hood and Allen, 1971; Sumida et al., 1972; Clemens et al., 1973; Dryden and Marchello, 1973; Gillis and Eskin, 1973; Garrett et al., 1976; Skelly et al., 1978; Westerling and Hedrick, 1979; Yoshimura and Namikawa, 1983; Marmer et al., 1984; Eichhorn et al., 1985; Eichhorn et al., 1986b; St. John et al., 1987; Larick et al., 1989; Mitchell et al., 1991; Khatri, 1992; Mills et al., 1992; Sturdivant et al., 1992).

The values reported by these investigators are in fairly close general agreement with values published by the National Live Stock And Meat Board (1990) (Table 35).

Influence of Fatty Acids on Flavor

Flavor investigations have been primarily conducted to determine palatability differences from forage versus grain fed beef. Hornstein (1971) reported fat may influence flavor in two ways: (1) fatty acids, upon oxidation, can produce carbonyl compounds that are potent flavor components and (2) fat
Table 35. Fatty acids as percent of total fat for retail cuts of beef

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Trimmed Retail Cuts</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>2.7</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>22.4</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>11.7</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>Other SFA</td>
<td>1.9</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>16:1</td>
<td>3.8</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>18:1</td>
<td>37.9</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>Other MUFA</td>
<td>1.9</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>18:2</td>
<td>3.2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>18:3</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>20:4</td>
<td>0.5</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Other PUFA</td>
<td>0.3</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Fatty Acids as Percent of Total Fat For Beef

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>38.7</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>43.6</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>4.1</td>
</tr>
</tbody>
</table>

may act as a storage depot for odiferous compounds which are released upon heating. Few studies have been published which relate qualitative or quantitative differences in beef fat flavor compounds to palatability.

Fatty acid composition can influence volatiles produced and serve as important flavor precursors since they are the primary source of carbonyl
compounds upon heating (Herz and Chang, 1970; Selke et al., 1977, 1980). The more unsaturated the fatty acid, the more susceptible it is to oxidation and a greater rate of autoxidation (Gokolp et al., 1983). Pearson et al., (1977) reported that the abundance of oxidatively unstable polyunsaturated fatty acids in animal tissues makes these highly susceptible to off flavors and in particular, warmed-over flavor, which is rapid flavor deterioration in cooked meats.

Considerable amounts of contradictory data have been reported concerning the influence of feed on flavor of beef. In general, most studies conclude that beef from cattle finished on low-energy forage diets has a less desirable flavor than that from cattle finished on high energy grain diets (Brown et al., 1979; Bowling et al., 1978; Davis et al., 1981; Dolezal et al., 1982a; Harrison et al., 1978; Hedrick et al., 1983; Larick and Turner, 1990; Melton et al., 1982 a,b; Reagan et al., 1977; Schroeder et al., 1980; Skelley et al., 1978; Smith et al., 1977; St. John et al., 1987; Tatum et al., 1980; Westerling and Hedrick, 1979). Undesirable beef flavor of forage fed beef has been characterized as grassy (Berry et al., 1980; Larick et al., 1987; Schroeder et al., 1980) gamey (Larick and Turner, 1990) or intense sour, milky-oily and fishy flavor (Brown et al., 1979; Melton et al., 1982b; Larick and Turner, 1990).

Compared with beef produced from grain diets, beef produced on grass pastures has different concentrations of several flavor precursors. Beef produced on grass pasture has lower amounts of glucose (Melton et al., 1982 a,b) and γ-tocopherol (Mann, 1983, as cited by Melton, 1990) and higher levels of caroteneoids and α-tocopherol (Mann 1983, as cited by Melton, 1990). However, the greatest sensory difference in beef from forage fed and grain fed beef seems to be in the flavor of fat (Meyer et al., 1960; Harrison et al., 1978; Melton, 1983;
Larick et al., 1987, 1989; Larick and Turner, 1989, 1990) Forage fed beef contains higher contents of saturated and omega-3 polyunsaturated fatty acids and lower levels of monounsaturated and omega-6 polyunsaturated fatty acids (Melton, 1982 a,b; Medeiros et al., 1987). Beef from grain fed cattle has less C15:0, C16:0, C18:0, C18:3, C20:0, C20:3, C20:4 and C20:5 compared with forage fed cattle (Brown et al., 1979; Miller et al., 1981; Melton et al., 1982a,b; Westerling and Hedrick, 1979; Larick and Turner 1990). Melton et al. (1982b) reported that C15:0, C18:0, C18:3 and C20:4 were positively correlated with the milky-oily, sour and fishy flavor characteristics.

Polyunsaturated fatty acid composition seems to be most affected by diet and are generally associated with the phospholipid fraction (Hornstein et al., 1961). In fact, Larick et al. (1989) found that as the concentration of phosphatidylethanolamine, phosphatidylcholine + lysophosphatidylethanolamine and lysophosphatidylcholine increased, there was an increase in sensory perception of ammonia, gamey, liverish and rotten flavor characteristics of steaks from Hereford, Brahman and Bison steers fed a grain diet. Therefore, higher concentrations of polyunsaturated fatty acids may be partially responsible for the higher intensity of undesirable flavors in certain types of beef (Melton, 1983) and also contribute to more rapid development of oxidative rancidity in forage fed beef (Reagan et al., 1977; Schroeder et al., 1980).

The source of increased contents of C18:2 and C18:3 in tissues from forage fed animals may be fatty acids that escape hydrogenation in the rumen prior to being absorbed and are deposited in adipose tissue. The mechanism resulting in increased levels of C20:3, C20:4 and C22:5 is unknown, although C18:2 N-6 is a precursor for various long chains polyunsaturated fatty acids in
the N-6 series, including C18:3, C20:3, C20:4, C22:4 and C22:5 (Crawford et al., 1976).

Studies (Larick et al., 1987; Varner et al., 1988) have been conducted to determine levels of flavor volatiles and correlate these with sensory evaluation of flavor. Bolton (1987) as cited by Melton (1990) reported that the levels of several volatiles were positively correlated with the intensity of cooked beef fat flavor (P<.05): diacetyl, 2, 3 pentadione, octane, hexanal, 1-hexanol, octanol and one unknown compound. Tolulene was negatively correlated with beef fat flavor intensity (r=-.44, P<.01). Varner et al. (1988) as reported by Melton (1990) found that pentanal and tolulene were important in eliciting flavor differences between forage and grain fed beef. Larick determined that levels of 13 volatiles were positively correlated with grass flavor intensity of both ground beef and steak (P<.01). The highest correlation coefficients were between flavor intensity and phyto-2-3ene. This is a degradation product of chlorophyll and is much higher in fat from cattle fed grain than forages (Melton, 1990).

**Effects of Cooking on Fat**

Heating meat has profound effects on fat. The texture becomes more soft or liquid and some fat may be lost, aromatic compounds are volatilized, and lipids may undergo chemical changes, such as hydrolysis and oxidation (Allen and Foegeding, 1981).

Fresh, uncooked meat was more stable during storage at 5–7°C when compared with cooked meats based based on thiobarbituric acid (TBA) values (Tims and Watts, 1958). Malonaldehyde content on a variety of retail muscle food products was determined before and after cooking by Siu and Draper
They found levels of malonaldehyde achieved after cooking were related to the species of meat, whether it was fresh or cured, and the cooking time. Lipid oxidation was minimal in cuts which were cooked in a short time. However, after an induction of 60–90 minutes during cooking, malonaldehyde concentrations in pork and beef roasts increased rapidly during the subsequent 60-90 minutes of cooking. Malonaldehyde values ranged from being two to ten times higher than before cooking for large roasts. However, steaks and chops cooked for a much shorter time showed no increase up to four times the amount of malonaldehyde as was observed before cooking. Differences in malonaldehyde values were greater in pork and chicken compared with beef, presumably due to differences in the quantity of polyunsaturated fatty acids, in which case beef is the lowest. Results reported by Huang and Greene (1978) were in agreement with those of Siu and Draper (1978). Beef cooked to high temperatures and/or for long periods of time developed lower TBA values than samples heated at lower temperatures for shorter periods of time. These results suggest that long-time, low temperature cookery, which is extensively used in preparing beef roasts for food service establishments, was not as stable to oxidative rancidity as roasts cooked in a more conventional manner.

Igene and Pearson (1979) and Igene et al. (1980) evaluated "warmed over flavor" in beef. They determined that total phospholipids, especially phosphatidylethanolamine, and their polyunsaturated fatty acids were the major factors responsible for warmed over flavor development. Phosphatidyl choline and triglycerides were less important. Cooking caused a greater reduction in polyunsaturated fatty acids of phosphatidylethanolamine compared with those of phosphatidylcholine, specifically C20:4.
Furthermore, flavor and aroma of cooked meats appear to be produced by a variety of complex reactions between lipids, amino acids and sugars (Mottram and Edwards, 1983; Salter et al., 1988). Phospholipids have an important effect on the nature of the volatiles from cooked meat by contributing aliphatic aldehydes and alcohols to the mixture of volatile components (Mottram and Edwards, 1983). Considerable evidence has revealed that phospholipids are major contributors to oxidative rancidity in meat, whereas triglycerides have only a minor role (Love and Pearson, 1971; Lee and Dawson, 1973; Igene and Pearson, 1979; Melton, 1983; Pikul et al., 1984). Previous studies have shown that heating meat caused hydrolysis of plasmalogens in the phospholipids, liberating fatty aldehydes which were subsequently recovered in the neutral lipid (Fogerty et al., 1989; 1990; 1991).

Evaluation of components influencing flavor desirability and stability are very important. With advances in laboratory technology and sophistication of research approaches, much knowledge can be gained in this complex area. This is important because lipids are important to the physical and chemical characteristics in muscle foods. They contribute to the species flavor associated with muscle foods, affect the potential lipid oxidation which influences shelf-life characteristics and may influence production of toxic substance formation via lipid oxidation which could be harmful to consumers ingesting oxidized muscle foods.

Effect of Anatomical Location on Fatty Acid Composition

It is necessary to clarify the lipid and fatty acid composition of adipose tissue systematically. Studies have been conducted which report fatty
composition of subcutaneous, intramuscular, intermuscular and perenephric fat.

Depot site differences (specifically differences in unsaturated/saturated ratios) were originally thought to be a function of the body temperature differential. However, temperature differential does not appear to be large enough to warrant these differences. Depot site differences may be due to rate of growth and development and purpose and function of individual depot (insulation, energy reserve, or both) (Terrell et al., 1969)

Eichhorn et al. (1985) evaluated fatty acid composition of total lipid extracts of muscle and adipose samples from crossbred bulls and steers. They found variation existed between different sampling sites (Table 36). Muscle samples from steers contained 51 to 63% unsaturated fatty acids. Perenephric adipose samples were relatively high in percentage of saturated fat, which comprises about 60% of the total lipid fatty acids. Samples from the triceps brachii and semitendinosus muscles of steers contained approximately 9% more unsaturated components than those from the longissimus. Semitendinosus muscle contained about 6% more PUFA than the longissimus, with the triceps brachii having an intermediate value. Adipose tissue samples had a very low percentage of PUFA. Arachidonic acid (C20:4), an essential fatty acid and a major precursor for prostaglandins was present primarily in muscle tissue, whereas adipose tissue contained only trace amounts.

Yoshimura and Namikawa (1983) determined fatty acid composition of subcutaneous fat at a constant slaughter weight from samples removed from the chuck, loin, rump, brisket and flank regions. With the exception of samples from the flank region which tended to be slightly more saturated
Table 36. Means for percentage composition of fatty acids in total lipid extracts of muscle and adipose tissue from crossbred steers

<table>
<thead>
<tr>
<th>Fatty Acid Component</th>
<th>Semitendinosus M.</th>
<th>Triceps brachii M.</th>
<th>Longissimus M.</th>
<th>Subcutaneous Adipose</th>
<th>Perinephric Adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.9</td>
<td>2.0</td>
<td>2.9</td>
<td>4.1</td>
<td>4.5</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.8</td>
<td>23.8</td>
<td>30.4</td>
<td>30.1</td>
<td>29.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>11.3</td>
<td>11.1</td>
<td>14.2</td>
<td>13.9</td>
<td>24.1</td>
</tr>
<tr>
<td>C16:1</td>
<td>5.0</td>
<td>5.6</td>
<td>4.5</td>
<td>6.2</td>
<td>3.9</td>
</tr>
<tr>
<td>C18:1</td>
<td>41.1</td>
<td>45.6</td>
<td>40.7</td>
<td>41.2</td>
<td>34.7</td>
</tr>
<tr>
<td>C18:2</td>
<td>8.1</td>
<td>6.4</td>
<td>4.1</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.0</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>C20:4</td>
<td>3.5</td>
<td>2.5</td>
<td>1.1</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Total SFA</td>
<td>38.6</td>
<td>37.6</td>
<td>48.3</td>
<td>49.6</td>
<td>58.7</td>
</tr>
<tr>
<td>Total UFA</td>
<td>60.8</td>
<td>62.2</td>
<td>51.4</td>
<td>50.0</td>
<td>41.1</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>12.6</td>
<td>9.7</td>
<td>5.8</td>
<td>2.5</td>
<td>2.3</td>
</tr>
</tbody>
</table>

(approximately 3%), differences among specific fatty acids at various locations seemed minimal.

Clemens et al. (1973) compared fatty acid composition of intramuscular fat removed from longissimus muscle and subcutaneous fat removed from the 12th rib region from bull and steer carcasses slaughtered at various ages. Results from cattle slaughtered at 15 months of age are reported in Table 37. These data would suggest that at a similar age, intramuscular fat contains a higher content of palmitic, stearic, and linoleic fatty acids and less palmitoleic and oleic fatty acids than subcutaneous adipose tissue. These data compared
Table 37. Fatty acid composition of subcutaneous and intramuscular fat from bulls and steers slaughtered at 15 months of age

<table>
<thead>
<tr>
<th>Tissue Fatty Acids (Relative %)</th>
<th>Fat Depot</th>
<th>14:0</th>
<th>14:1</th>
<th>16:0</th>
<th>16:1</th>
<th>17:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td></td>
<td>4.3</td>
<td>2.3</td>
<td>22.6</td>
<td>6.7</td>
<td>1.5</td>
<td>9.4</td>
<td>51.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Intramuscular</td>
<td></td>
<td>2.2</td>
<td>0.7</td>
<td>30.3</td>
<td>2.9</td>
<td>0.8</td>
<td>17.5</td>
<td>37.3</td>
<td>9.5</td>
</tr>
</tbody>
</table>

favorably with Gillis and Eskin (1973) and Westerling and Hedrick (1979) who reported intramuscular fat contained less oleic and more linoleic acid than subcutaneous fat covering the semitendinosus, transverse abdominisus and triceps brachii muscles. Effect of depot site on fatty acid composition revealed that the outside triceps brachii had the highest percentage of C16:1 and C18:1 while seam fat had the lowest percentages of these fatty acids. The reverse was true for C18:0. The fact that the seam had the smallest percentage of C18:0 and C16:1, yet the largest percentage of C18:0 accounts for its relative degree of hardness and suggests that seam fat may have a different metabolic purpose compared with that of subcutaneous depots. Outer layers of subcutaneous fat had a larger percentage of C16:1 and C18:1 than respective inner layers. The outer and inner layers of the triceps brachii were the most unsaturated depots observed.

O'Keefe et al. (1968) compared fatty acid composition at three locations within beef carcass musculature. They found significantly more C14:0 in longissimus muscle than in semitendinosus. However, the effect was not large enough to cause a significant difference in the total amount of saturated fatty acids found in the two muscles. Furthermore, Dryden and Marchello (1970) determined fatty acid composition on longissimus, semimembranosus, and
triceps brachii. They reported no significant differences related to amounts of specific fatty acids among these muscles. These results were in agreement with those reported by Garrett et al. (1976) for the same three muscles. In addition, Garrett and co-workers (1976) found that omental and kidney fat had lower percentages of C16:1 and C18:1 and higher percentages of C18:0 than fat from the rib or tailhead.

St. John et al. (1987) evaluated fatty composition of beef steer longissimus and semimembranosus muscle along with overlying subcutaneous fat corresponding to each muscle and perirenal fat. They found perirenal adipose tissue in steers contained more C18:1 than subcutaneous fat covering the semimembranosus or longissimus. Both anatomical locations of subcutaneous fat exhibited similar fatty acid profiles. Longissimus muscle had less C16:1 (1.4 versus 2.5 percent) and C18:2 (3.2 versus 4.8 percent) than semimembranosus muscle. No other differences were observed relative to the two muscles sampled. Subcutaneous adipose tissue, however, had more C18:0 and less C16:0 when compared with muscle tissue. These differences were larger for the perirenal fat.

Mitchell et al. (1991) compared fatty acid composition of beef longissimus and gluteus medius muscle and overlying subcutaneous fat. Longissimus muscle had more C14:0 and C15:0 compared with the gluteus medius. Differences were not apparent between locations for adipose tissue. However, adipose tissue had a higher content of C18:1 than muscle tissue.

Sturdivant et al. (1991) reported percentages of major fatty acids in 1/2 to 7/8 crossbred Wagyu steers for various depot sites. They found subcutaneous fat had a higher content of C14:1 and C16:1 and had less C18:0 and C18:2 than
intramuscular fat or longissimus muscle. Interestingly, intramuscular fat had a higher content of C18:0 than subcutaneous or longissimus muscle. This same group (Sturdivant et al., 1991) also determined fatty acid composition on purebred Black Wagyu cattle for these same depot sites. They found longissimus muscle had more C14:0 and C16:0 and less C18:1 than intramuscular or subcutaneous fat. The purebred cattle had more C18:1 (4 to 8 percent) and less C16:0 (4 to 6 percent than their crossbred counterparts. Furthermore, Waldman and coworkers (1968) evaluated effect of locations on fatty acid profiles by comparing outside and inside subcutaneous fat, intermuscular fat from the chuck and intramuscular fat from the longissimus muscle. Their results of subcutaneous fatty acid means indicated that total concentration of fatty acid increased from external to internal sample locations. This observation was primarily linked to a replacement of C18:1 by C18:0 in internal fat depot and to a lesser extent to a replacement of C16:1 by C16. The chuck intermuscular fat had more C18:0 (6%) and less C18:1 (4%) compared with subcutaneous fat. Longissimus muscle had a fatty acid profile which compared similarly to subcutaneous fat although, C16:0 was 3% higher and C18:1 was 3% lower in longissimus than subcutaneous. Marmer et al. (1984) compared fatty acid composition from various tissue sites. They analyzed longissimus, semitendinosus and psoas major, along with subcutaneous fat from the twelfth rib region and perirenal fat. For muscle tissue, they reported that the psoas major contained more saturated fat than longissimus or semitendinosus. Most of this was attributed to C:18:0. The semitendinosis contained the highest content of C18:1. Furthermore, this group reported that perinephric fat was more saturated than subcutaneous fat. This was attributed
to a considerably higher content of C18 (12.5 versus 24.7%) for perirenal fat compared with subcutaneous.

Westerling and Hedrick (1979) analyzed effect of depot site on fatty acid composition of longissimus muscle and overlying subcutaneous fat. They reported that subcutaneous fat contained more (p<.05) C16:0 and C18:1 and less C18:2, C18:3 and C20:4 than intramuscular fat from the longissimus. Eichhorn et al. (1985) compared lipid extracts from semitendinosus, triceps brachii and longissimus muscle and its overlying subcutaneous fat and a sample of perirenal fat. They reported that the longissimus muscle lipid extract was 9 percent more saturated than the triceps brachii or semitendinosus. This was due to longissimus having more C16:0 and C18:0. The subcutaneous fat was less saturated than the perirenal fat mainly because of a reduction in C18 and an increase in C18:1 compared with perirenal adipose tissue. Yoshimura and Namikawa (1983) compared fatty acid composition from subcutaneous fat samples excised from the chuck, loin, rump, brisket and flank. Of these five locations, flank subcutaneous fat contained the highest percentage of C16:0 (p<.01) and C18:0(p<.05) and resulted in the lowest percentage of total unsaturated fatty acids. Hood and Allen (1971) compared fatty acid composition of lipid extracts from longissimus muscle and its overlying subcutaneous fat. They reported that subcutaneous fat had more C16:1 and C18:1 and less C18:0 than lipid from the longissimus muscle.

Link et al. (1970) reported that changes in relative amounts of intramuscular C14:0, C16:0, C17:0 and C18:1 fatty acids from longissimus muscle were related to season of the year. They found a change in relative amounts of these fatty acids occurred between March and May. The total amount of
unsaturated fatty acids was lowest during winter and highest during spring and early summer. Seasonal differences noted in intramuscular fatty acid composition may be due to the animals response to changes in ambient temperature. Marchello et al. (1967) suggested that a cold environment may act as a stimulus to enhance a dehydrogenase enzyme system which would increase the biosynthesis of unsaturated fatty acids. Conversely, heat might act to inhibit a dehydrogenating system. In addition, solubility properties of fatty acids are such that the presence of a double bond is equivalent to removing two carbon atoms. The two major fatty acids of beef lipids, C16 and C18:1, are a pair of fatty acids with similar physical properties. Both of these fatty acids were significantly affected (p<.01) by season. The simple correlation between these two fatty acids of -.85 (p<.01) indicated a possible replacement of one for the other. However, it is possible alternative routes of fatty acid synthesis are responsible for producing differences in these fatty acids (Link et al., 1970). Yoshimura and Namikawa (1983) suggested that since adipose tissue is the major site of fatty acid synthesis in ruminants (Allen et al., 1976), and the principal fatty acids synthesized in bovine adipose tissue were C16, C18 and C18:1, differences in fatty acid composition may be due to the endogenous fatty acid mechanism including desaturation and chain elongation. Several researchers have reported the fatter and older an animal becomes, the more monounsaturated their depot fat becomes (Waldman et al., 1968; Link et al., 1970; Clemens et al., 1973; Westerling and Hedrick, 1979; Mills et al., 1991; Sturdivant et al., 1992). Elevated monounsaturated fatty acids in beef adipose tissue in mature cattle may parallel what Sturdivant et al. (1992) observed in the Wagyu breed. They postulated that the increases observed could have
resulted from increased absorption of the dietary monounsaturated fatty acids or elevated activity of stearoyl-CoA desaturase with adipose tissue. Influences of absorbed monounsaturated fatty acids are unlikely, however, because C14:1 and C16:1 are not plentiful in feeds and those ingested would be largely hydrogenated to the saturated fatty acids by ruminal microflora. Because stearoyl-CoA catalyzes the conversion of all saturated fatty acids to n-9 monounsaturated fatty acids, this single enzyme could be responsible for elevated monounsaturated fatty acids.

**Gender**

Fatty acid composition of beef adipose tissue has been shown to be influenced by sex of the animal when steers and heifers were compared (Terrell et al., 1968; Waldman et al., 1968; Terrell et al., 1969; Link et al., 1979 a,b; Westerling and Hedrick, 1979; Yoshimura and Namikawa, 1983). These differences showed that steers had a slightly larger proportion of saturated fatty acids than heifers due mainly to higher contents of C14:0, C16:0 and C18:0. Westerling and Hedrick also reported steers had a higher content of C18:2 and C20:4. Heifers generally had higher content of C18:1 than steers. The magnitude of difference is generally small, when comparing specific fatty acids from steer and heifer adipose tissue. Hood and Allen (1971) found no difference when comparing steer and heifer fatty acid composition.

Fewer studies have been conducted which investigate differences between bull and steer fatty acid composition. Hood and Allen (1971) found higher fractions of C18:0 and C18:2 and less C16:0 and C18:1 in the intramuscular lipid of bulls when compared with steers. This agrees with Gillis
and Eskin (1973) who found bulls had higher contents of C14:0, C16:1 and C18:2 and less C18:1 with a similar amount of C16:0 and C18:0. Clemens et al. (1973) reported only two fatty acids were different for bulls compared with steers. Their data indicated that C16:0 was higher and C17:0 was lower for steers than bulls. No other differences were attributable to castration. More recently, Eichhorn et al. (1985) reported less C16:0 and higher C18:2 and C18:3 for bulls than steers in lipid fatty acids of longissimus muscle. However, Khatri (1992), found no significant difference due to sex (bull or steer) for nine fatty acids analyzed.

Fatty acid compositional differences observed for gender that have been reported may be due to the possible effect of sex hormones on enzyme systems such as desaturase. It may also be possible that the androgens produced by the intact male stimulate fat catabolism and intricate relationships between various hormones and enzymatic systems could account for compositional differences in intramuscular lipid of bulls and steers (Gillis and Eskin, 1973). Eichhorn et al. (1985) offers a different explanation as to changes in fatty acid composition for bulls compared with steers based on their results. They found the triacylglycerol:phospholipid ratio is the major factor which determines fatty acid composition in muscle. Triacylglycerol: phospholipid ratio reflects fat:lean ratio, which is strongly influenced by degree of marbling in muscle. Adipose tissue consists mainly of triacylglycerols which have a relatively high percentage of saturated fatty acids, whereas most polyunsaturated fatty acids are present as phospholipids in muscle cell membranes. Therefore, additional marbling deposition in the carcass due to castration increases percentages of
saturated fatty acids in steers and decreases polyunsaturated fatty acid content compared with bulls.

**Beef Quality Grades and Grading**

**History**

Producers, packers, processors and distributors are cognizant of live animal and meat grades. Market news reports utilize federal grade nomenclature in reporting prices, and within the meat trade, both federal and private (brands) grades are used in wholesale transactions. Consumers are aware of federal grades—though most possess little knowledge about grades. (Hutchinson, 1970).

The purpose of grading is to segregate units of a commodity into lots, or groupings, which have a high degree of uniformity in certain specified attributes associated with market preferences and valuation (McCoy, 1981).

Beef grades have provisions to estimate both quality and cutability. Quality grades in beef are determined on subjective considerations related to palatability indicating characteristics. However, yield grades for beef are determined primarily by objective measurements. Thus, it can be seen that even though the definition of grading is straightforward, drawing up standards or specifications for a system of grades is a complex problem (McCoy, 1981).

Some attributes upon which grades are based can be evaluated directly, others indirectly, through so-called indicators. Therefore, tremendous amounts of research and consultation is involved in developing grade standards.
The objectives of a company in setting up private grades are only partially compatible with objectives of a public grading system. One primary concern of a private firm is a grading system which adequately describes the quality/quantity of the product so that trade can be conducted with a minimum of time, effort and expense. Grade designations which consistently and fully describe a product do away with the necessity of personal inspection by buyers. Transactions can be accomplished by mail or phone which decreases expenses incurred by both buyers and sellers. In this respect, private and public grading have a common purpose. However, private firms may also use grades to differentiate their products from those of their competitors (i.e. Certified Angus Beef compared with USDA Choice).

From the standpoint of public interest, grades and grade standards are confusing. Without some official supervision of application of specified standards, suspicions may arise as to whether products meet expected standards.

Producers' interests are associated with the degree to which grade usage assists them in obtaining equitable prices for their products. Consumers primarily are interested in obtaining products which are consistent with their preferences and the prices they pay.

In view of these considerations, it was considered to be in the public's best interest to inaugurate a system of federal grades and grading. However, this does not exclude individual firms from using private grades, either alone or in combination with federal grades.

At the turn of this century, a variety of market terms evolved in particular regions which were more or less descriptive of certain quality characteristics of beef cattle native to these regions. Associated with expansion
of the large terminal markets of that era was the private publication of market prices using local class and quality information. This was a significant advancement over no published prices at all. These reports were useful to regional livestock and meat trade influences. Although, several weaknesses were apparent and there was a lack of uniformity in terminology and standards among markets. A price quotation on "native cattle" for example, varied from one market to the next, or when some markets did not have a classification of "native cattle."

In many cases an accepted term was not used uniformly from person to person, or throughout the years at a given market. These conditions were symptomatic of growing pains in any developing industry/economy. Trade was hindered to the extent that buyers had to physically inspect all livestock/carcasses/wholesale cuts.

Shortcomings of this situation were evident for many years and efforts were undertaken to develop uniform grades and standards. The Agricultural Experiment Station of Illinois was a leader in this area. Under the direction of professors Mumford and Hall who conducted several studies between 1900 and 1908, the Department of Agriculture eventually based its development of the standards for grades of carcass beef. Although, these studies did not stimulate immediate governmental action, a movement developed for official market news reporting. Ensuing debates over implementation of market reporting could not be separated from consideration of official grades because the lack of uniformity in local grade names and laxity in local grade specifications rendered them unsatisfactory for market reporting purposes. In response to this need, USDA established an Office of Markets in 1913. That office began
work in the development of official grades for live animals and meat about 1915 (Dowell and Bjorka, 1941). Research conducted at Illinois provided a starting point, however, vested interests in local grades and lack of agreement on grade standards were serious obstacles in obtaining a consensus on uniform grades. Nevertheless, unofficial and unpublished grade specifications were drawn up and used for market reporting of meat prices beginning in 1916.

The first grades were formulated for dressed beef in 1916. They provided the basis for uniformly reporting dressed beef markets according to grades. These were initiated as a national service program in 1917 (USDA, 1965) and these beef grades were used in unofficial, tentative status in 1926.

When evaluating initial grade standards, essentially there were four objectives from which the grade standards seemed to have evolved. They were: 1) characteristics–in terms of inherent attributes, and internal and external components–had to be identified that facilitated the uniform and consistent segregation of carcasses; 2) a method or methods to quickly and accurately measure the various characteristics had to be developed; 3) a set of grade names had to be developed; 4) a functional nationwide program had to incorporate the first three objectives (May, 1981).

Characteristics used in segregating carcasses had to meet certain criteria: They had to: 1) always be present, 2) be inherent in the carcass and not subject to sudden or material changes and 3) be detected and appraised readily. Consequently, on June 3, 1926 the Official United States Standards for Grades of Carcass Beef were based on three factors. These were conformation, finish and quality.
The major impetus behind initiating these standards was the Better Beef Association which was formulated from three breed associations. These were the American Aberdeen-Angus Breeders' Association, American Shorthorn Breeders' Association and American Hereford Cattle Breeders' Association. The Better Beef Association mediated concerns and eventually achieved agreement between cattle producers and beef packers and requested voluntary grading and marketing of the top two beef grades (Kiehl and Rhodes, 1960).

Federal beef carcass grading was officially begun on May 2, 1927. This was done on an experimental basis for one year at no cost to the packer and only Choice and Prime carcasses were graded. Initial acceptance was not easily forthcoming as the "big five" packers had initiated carcass sorting, selection and sales of carcass beef using their own house brand specifications.

Beef grading has been performed by federal graders on a voluntary basis except during World War II and the Korean Conflict when it was mandatory for all federally inspected plants converting cattle to beef.

Initial grading standards were based on three factors. These are conformation, finish and quality. Conformation was the shape, build, outline or contour of the carcass. Finish referred to the quantity, character, quality and distribution of the fat. Quality referred to a characteristic of the flesh or lean meat of the carcass and of the intermuscular and intercellular fat contained therein. Furthermore, subsequent definitions for quality included the thickness, firmness and strength of both the muscle fiber and the connective tissue. It involved the quantity, consistency and character of the juices that surrounded and permeated the muscle fiber and the connective tissue. High quality meat possessed well-developed, firm, muscular tissue with a minimum
of strength in the fiber and connective tissue. It had a high proportion of juice to dry fiber, and the juice was of such consistency that the flesh remained firm and resilient when chilled. Thus, the combined factors, conformation, finish and quality consistently and uniformly segregated carcass beef according to established grade categories and uses of grades at that time (May 1981). An example of the 1926 grade description for a steer beef carcass, Choice or No. 1 is as follows:

**Choice or No. 1:** A Choice or No. 1 grade steer beef carcass has an excellent conformation, finish, and quality. Rounds, loins and ribs are well-developed and plump. Chucks and plates are very thick and heavily fleshed. The neck is short and thick. Shanks are short and well-muscled. The exterior fat covering generally is smooth but may be slightly wavy. Cod, crotch, kidney and other fats may be slightly less or more than required for the ideal carcass. Such fats are of excellent quality, being firm, brittle and creamy white. The cartilages on the chine and breast bones, especially in young steer carcasses, are pearly white, but may be slightly ossified. The bone may be soft and red, or slightly hardened, and of grayish-white color, especially if the animal was nearing 4 years of age. The flesh is firm, velvety and of an attractive light or cherry-red color. Marbling is always present in the loins, ribs, rounds and chucks.

In 1926 these descriptions were considered a progressive step. However, many of these criteria have been classified as ambiguous, subjective, complicated and unrealistic. Considerable input from many parties and supportive research conducted by the scientific community have enabled the foundation for updating initial grade standards. Present standards differ from original ones in several ways, the most significant being that the grade of a beef carcass now consists of separate evaluations of two general considerations: carcass cutability (yield grade) which is a prediction of closely trimmed boneless retail cuts which can be fabricated from a carcass, and the palatability indicating characteristics of the lean (quality grade) which primarily include degree of
marbling as evaluated in the ribeye between the 12th and 13th rib, and assessment of skeletal and lean maturity. Descriptions for these quality traits are subjective whereas the yield grade is based on objectivity. The quality grade descriptions are much more definitive than the original ones (May, 1981).

Effect of Length-of-Feed on Meat Quality and Palatability

USDA beef quality grades are the most accepted and utilized standards the beef industry has to account for differences in cooked beef palatability. USDA quality grades are extremely authoritative, and many management and marketing decisions are based on them. The majority of cattle feeders feed specific diets and utilize certain management practices to produce one product – USDA Choice beef.

Time on feed itself may have some affect on meat quality and palatability. With an increase in time-on-feed comes an increase in total carcass lipid (Marchello et al., 1976). Additionally, increased time-on-feed was associated with increased carcass maturity (Zinn et al., 1970a; Tatum et al., 1980). However, Zinn et al. (1970a) reported that it was not until after 180 days on feed that animal age exerted the greater negative influence on tenderness.

May et al. (1992) fed Angus and Hereford steers as high concentrate diet from 0 to 196 days. Steers were 16 months old at beginning of the feeding period and were slaughtered every 28 days until the end of the feeding trial. Taste panel tenderness, amount of perceived connective tissue and shear force value peaks at 112 days were slightly less desirable for cattle fed longer than 112 days.
Miller et al. (1987) fed steers a high energy concentrate diet for 0, 56, 112 or 168 days. All steers were slaughtered at 20 months of age. Tenderness of longissimus muscle and semimembranosus muscle steak increased with time on feed. However, the greatest improvement was observed from 0 to 56 days.

Matulis et al. (1987), using cull cows fed a high energy diet for 0, 28, 56, and 84 days, reported that marbling scores and quality grades improved significantly between 28 and 56 days. Carcasses from open and calved heifers were higher in quality after 42 days of high concentrate feeding than carcasses from 7 and 21 days on feed (Bond et al., 1986). Tatum et al. (1980) found that increased time on feed was associated with an increased marbling deposition.

Zinn et al. (1970b) and Dolezal et al. (1982b) fed cattle for 150 days to 270 days and 90 days to 200 days, respectively, and found that quality grades and marbling scores were highest (p<.05) for the longer times-on-feed. Zinn et al. (1970b) found that the deposition of intramuscular fat was not a continuous process, but it occurred at 60 to 90 d intervals.

No improvement in Warner-Bratzler shear (WBS) values were observed for steaks from longissimus dorsi (LD) muscles due to increased time-on-feed (Marchello et al., 1976). Tatum et al. (1980) found that WBS values generally decreased as degree of marbling increased, which resulted from a longer time-on-feed. However, this was not a linear relationship, nor was there always statistical significance in decreasing WBS force with increasing degree of marbling (Tatum et al., 1980). Matulis et al. (1987) also reported that WBS values of longissimus muscle decreased (p<.05) with increased time on feed. Similarly, animals fed 100 days or more had lower (p<.05) longissimus muscle WBS values than those animals fed for less than 90 d (Dolezal et al., 1982b).
Similar results were reported by Gutowski et al. (1979) who found WBS values were lower \( (p<.05) \) for long-fed cattle (98 d) than for short-fed cattle (48 d). However, WBS values were higher at 240 and 270 days than at 150, 180, and 210 days on feed (Zinn et al., 1970a), indicating that extremely long feeding periods, or increased animal age are detrimental to tenderness. Short feeding periods of 7, 21, and 42 days postpartum of once-calved heifers did not greatly influence palatability characteristics (Bond et al., 1986). While some studies have concluded that feeding an energy-rich diet prior to slaughter will improve palatability (Tatum et al., 1980; Dolezal et al., 1982b), others have observed diminutive advantages or negative effects from extensive feeding periods (Zinn et al., 1970a). Tatum et al. (1980) and Dolezal et al. (1982b) concluded that the length of feeding required to obtain the desired flavor, juiciness and tenderness of beef seemed to be optimal at approximately 100 days for yearling cattle fed a normal finishing diet.

**Effect of Preslaughter Growth Rate on Meat Palatability**

The amount of fat on retail beef cuts has been reduced drastically in the past few years due to changes in consumer demand. Because of this, animals are being selected with less external fat. Partly responsible for variations in tenderness is the decrease in fat cover of beef carcasses which had been theorized by Bowling et al., (1977); and Dolezal et al., (1982b).

Growth rate of cattle before slaughter has an important effect on tenderness (Aberle et al., 1981). These authors suggested that a relationship between growth rate and tenderness may be due to amounts, or activity, of endogenous proteolytic enzymes present at time of slaughter. Therefore,
preslaughter growth rate could be a more important indicator of meat
tenderness than length of time a high-energy diet is fed. This would be
especially true if the high-energy diet has been fed for some minimum period
of time to permit maximum activity of all enzyme systems which operate in
rapidly growing muscle tissue.

Cattle fed high-energy diets grow faster, have higher quality grades, may
have increased rates of protein turnover, have more soluble collagen, and have
more myofibril fragmentation. (Aberle et al., 1981; Wu et al., 1981; Hall and
Hunt, 1982; Miller et al., 1983). Fishell et al. (1985) reported that when steers
were fed a high-, medium-, or low-energy diet to obtain an average daily gain
(ADG) of 1.42, .77, and .34 kg per days, respectively, that sensory panel
tenderness scores were highest (p<.05) for the high ADG steers. However, there
were no significant differences in longissimus muscle WBS force values for
different rates of ADG, although the high ADG steers tended to have lower
WBS values (Fishell et al., 1985). Several researchers have reported increased
growth rate before slaughter was accompanied by increased collagen synthesis
and turnover (Wu et al., 1981; Miller et al., 1983; Fishell et al., 1985). Bailey
(1985) reported that as growth rate decreased, collagen cross-links stabilized and
the more stabilized cross-links resulted in less tender meat. Therefore, it is not
the amount of collagen present, but the quality of the collagen that contributes
to meat texture (Bailey, 1985). However, Hall and Hunt (1982) reported steers
slaughtered while in the A-maturity age range which had tolerated wide ranges
in feeding regimen, had little affect on tenderness, amounts of total collagen or
collagen solubility. Accelerated production in which steers are fed a high-
energy diet beginning after weaning and slaughtered at a Good-grade (now
Select) endpoint, resulted in longissimus muscle palatability at least equal to palatability of conventionally-produced steers (Dikeman et al., 1985).

Effect of Subcutaneous Fat Thickness on Meat Palatability

Increased subcutaneous fat depth is one consequence of feeding a high energy diet for long periods (Dolezal et al., 1982a). The beef industry needs to produce beef with less fat in order to meet the growing consumer demand for lean beef (Riley et al., 1983a). Bowling et al. (1977) reported moderate levels of subcutaneous fat reduce the rate of carcass temperature decline during postmortem chilling and improve beef tenderness by lessening the extent of cold-induced toughening and by enhancing rate and extent of postmortem muscle autolysis. For these reasons, subcutaneous fat thickness maybe one of the most important factors affecting palatability. Dikeman et al. (1979) found that WBS values were significantly higher for rib steaks exhibiting less that .64 cm of fat thickness than for rib steaks possessing at least .64 cm of subcutaneous fat cover. Select grade, non-electrically stimulated steers with at least 7.6 mm of subcutaneous fat produced steak which did not differ significantly from USDA Select and Standard grade, non-electrically stimulated steers with less than 7.6 mm of fat cover for juiciness, muscle-fiber tenderness, conective-tissue amount, overall-tenderness scores, WBS force values, and cooking losses (Riley et al., 1983b). Additionally, electrically stimulated, young bull carcasses with at least 7.6 mm of fat did not differ (p>.05) from carcasses with less than 7.6 mm of fat for any palatability trait except connective-tissue amount. Non-electrically stimulated bull carcasses with at least 7.6 mm of fat had higher (p<.05) muscle fiber tenderness, overall tenderness, and juiciness ratings, and lower (p<.05)
WBS values than bulls with less than 7.6 mm of fat. Also, no differences (p>.05) in connective-tissue amount and cooking loss for the non-electrically stimulated bull carcasses of different fat thicknesses were observed (Riley et al., 1983b). Similar results were reported by Riley et al. (1983a) in which electrically-stimulated bull carcasses were stratified according to four fat thicknesses (less than 3.8 mm, 3.9 to 6.4 mm, 6.5 to 9.0 mm, and greater than 9.0 mm). No differences were observed in any palatability trait with the exception of connective-tissue amount, which was significantly higher in the 3.9 to 6.4 mm fat thickness group. However, non-electrically stimulated bulls from the 6.5 to 9.0 and greater than 9.0 mm fat thickness groups had higher (p<.05) muscle fiber tenderness and overall tenderness ratings and lower (p<.05) values than the other fat thickness groups. While there were no (p>.05) differences in connective-tissue amount among any of the fat thickness groups, the group with greater than 9.0 mm fat thickness did have higher (p<.05) juiciness ratings and lower (p<.05) cooking losses than any of the other groups, which did not differ significantly. Jennings et al. (1978) found that there were no significant differences in palatability traits or WBS force values between carcasses of less than 1.02 cm or greater than 1.52 cm of subcutaneous fat. Similar results were reported by Dikeman and Crouse (1975) that indicated carcass fat and longissimus intramuscular fat were unsatisfactory indicators of palatability and that increased carcass fat did not result in any measurable increase in palatability. Jennings et al. (1978) reported that it is possible that the .93 cm average value for fat thickness on carcasses selected to have less than 1.02 cm of fat thickness may have been sufficient to prevent cold-shortening induced toughness and juiciness problems under normal carcass chilling procedures.
Generally, research has shown that approximately 7.6 mm of subcutaneous fat is adequate to retard the severity of postmortem chilling and maintain acceptable product palatability. Subcutaneous fat in excess of 7.6 mm has little or no effect on meat palatability and WBS force values. Reports by Dolezal et al. (1982a) indicated that palatability of cooked beef increased as fat thickness increased from less than 2.5 mm up to 7.6 mm, but increases greater than 7.6 mm did not further improve palatability. Additionally, Tatum et al. (1982) reported that fat thickness levels of 7.6 to 10.2 mm provided relatively high assurance of desirable palatability of beef rib steaks. They further concluded that a minimum subcutaneous fat depth of 7.6 mm combined with a minimum of slight marbling were superior in palatability compared with steaks from carcasses which had Choice marbling and less than 7.6 mm of fat.

Stender (1984) stratified steer carcasses according to five levels of thicknesses, ranging from 0.3 cm to 1.55 cm average fat thickness, to determine the effect of fat thickness differences on palatability of beef rib steaks. No significant differences were found in any of the sensory attributes of steaks from steer carcasses between the average fat thickness levels of 0.56, 0.79, 1.04 and 1.55 cm fat. Steaks from carcasses of the group with 0.3 cm average fat were significantly (p<.05) less tender in addition to having less desirable flavor and overall acceptability than steaks from the higher fat thickness levels. Furthermore, Dikeman (1987) found similar trends when evaluating palatability attributes of 759 cattle used in the Germ Plasm Evaluation Project (U.S. Meat Animal Research Center, Clay Center, NE and Kansas State University, Manhattan). They found rib steaks from carcasses possessing less than 0.64 cm of fat had lower sensory panel tenderness and flavor scores and...
higher Warner-Bratzler shear force values than steaks from carcasses that had between 0.65 to 1.27 cm, 1.28 to 1.91 cm, 1.92 to 2.54 cm or those with greater than 2.54 cm.

**Effect of Physiological Maturity on Meat Palatability**

Carcass maturity is one of two subjective evaluations utilized by USDA graders to determine USDA quality grades of beef carcasses. Youthful carcasses have been reported to be more tender than the most mature carcasses (Simone et al., 1959; Tuma et al., 1962a; Goll et al., 1965; Romans et al., 1965; Breidenstein et al., 1968; Prost et al., 1975a,b; Cross et al., 1984; Smith et al., 1988). However, other researchers have found that carcass maturity had no significant effect on palatability (Norris et al., 1971; Berry et al., 1974; Carroll et al., 1976 Reagan et al., 1976; Miller et al., 1983).

Tuma et al. (1962a) reported that the greatest difference in tenderness of rib steaks was between 18 and 42 mo of age. However, these researchers found no significant differences for juiciness and flavor due to age. Simone et al. (1959) reported that sensory panel evaluations for tenderness, juiciness, and flavor traits in roast beef was higher from 18- than from 30-mo old steers. Romans et al. (1965) concluded that after evaluating steaks from A, B, C and D maturity carcasses that A and B maturity groups were significantly more tender than D-maturity steaks. However, these investigators found no significant difference between A, B, C and D maturity rib steaks for juiciness or WBS force. Additionally, B-maturity rib steaks were significantly more flavorful than D-maturity steaks. Breidenstein et al. (1968) observed that rib steaks from E maturity carcasses were less tender than those from A and B maturity carcasses.
These researchers, however, found no significant differences in flavor and juiciness among the three maturity groups. Goll et al. (1965) studied rib steaks from A-, B- and F-maturity carcasses and found that steaks from A and B maturity carcasses were more tender than those from F-maturity carcasses. However, these researchers did not observe any significant differences in juiciness and flavor due to maturity. Miller et al. (1983b) reported that there were no significant differences between A or B and C or D maturity carcasses for tenderness, juiciness, connective tissue amount, flavor desirability or WBS values. Norris et al. (1971) reported that carcasses representing three physiological maturity levels, ranging from young A to old B maturity, had little effect on palatability of steaks. Powell (1991) found that longissimus muscle from electrically stimulated carcasses from either 18 or 54 months of age cattle were similar after 3 weeks of postmortem storage to those non-electrically stimulated. Additionally, Carroll et al. (1976) found that within A maturity, a third of a degree of difference in maturity had no consistent influence on palatability whereas Seideman et al. (1984) reported steaks from 18 month old steers were more tender than those from 12 or 15 months old. Reagan et al. (1976) reported cattle ranging in age from 305 to 982 d did not differ significantly in fat percentage, WBS value, or sensory panel juiciness, tenderness and amount of connective tissue.

Shorthose and Harris (1990) recently conducted an investigation to determine tenderness of 12 beef muscles from animals of 8 different age groups ranging from 1 to about 60 months of age. The mean tenderness of these 12 muscles decreased significantly (p<0.001) with age. The rate of increase in toughness of individual muscles with animal age was related to their
connective tissue strength. Psoas major muscles were almost unaffected by increasing animal age, whereas high connective tissue strength muscles such as the biceps femoris tripled in toughness. Some muscles, such as the longissimus, gluteus medius and psoas major were acceptable in tenderness up to 48 months. Others, such as the gracillus and adductor became unacceptably tough as the animal age increased above two years of age, while the biceps femoris, deep pectoral and semitendinosus were unacceptably tough in animals younger than two years.

These investigators (Shorthose and Harris, 1990) also stated that it is important when attempting to measure the tenderness of a carcass to know which is the most representative, or index, muscle to sample. A comparison of their results suggests that psoas major, gracillus and longissimus should not be used. These muscles are less predictive of overall carcass tenderness than the semitendinosis, biceps femoris, semimembranosus and gluteus medius. This may be particularly important as we in the United States generally opt to perform tenderness evaluations on longissimus muscle and perhaps should broaden our outlook to include other muscles which may be more predictive of overall carcass tenderness.

**Effect of Marbling and USDA Quality Grade on Meat Palatability**

Characteristics of muscle, such as marbling, are included in meat grading standards of the U.S. Department of Agriculture as indicators of subsequent eating quality of cooked meat. Of the palatability traits of meat, tenderness, has more influence on the overall acceptability of meat than flavor or juiciness according to Norris et al. (1971) and Campion et al. (1975). Campion et al. (1975)
suggested that 2.9% fat in the longissimus muscle was sufficient for acceptability of cooked meat. This percentage corresponds to the slight degree of marbling in the USDA grading standards.

For a comprehensive overview of beef quality grades and its relationship to steak palatability, Parrish (1981) provides an excellent review as part of the National Beef Grading Conference. Jost et al. (1983) reported that marbling alone accounted for only 0.4% of the tenderness variation of the LD muscle. Armbruster et al. (1983) reported that marbling scores ranging from slight to extremely abundant explained less than 1.2% of the variation on tenderness and little of the variation in other sensory attributes. Smith et al. (1984) concluded differences in marbling (practically devoid to moderately abundant) explained about 33% of the variation in overall palatability ratings of loin steaks from A, B, C, and A+B maturity carcasses. Campion et al. (1975) reported that components of quality grade accounted for no more that 10% of the variation in any of the taste panel measurements, with marbling score accounting for the greatest portion of that variation.

Tatum et al. (1982) reported that marbling had a low, but positive relationship to all of the palatability traits of beef. More than 90% of the steaks with slight or higher degrees of marbling were desirable in overall tenderness, flavor, and overall palatability. Dikeman et al. (1979) found that taste panel tenderness scores, for steaks ranging in marbling from practically devoid to greater than moderate plus, were lowest (p<.05) for practically devoid and traces marbling and highest (p<.05) for modest average and above marbled steaks. In addition, flavor scores generally were higher for steaks possessing small minus or higher marbling than steaks displaying slight plus or less marbling. Also,
these researchers reported slightly abundant or more marbling produced juicier steaks than those ranging from slight minus to modest plus, which in turn were juicier than those possessing practically devoid and traces marbling. Breidenstein et al. (1968) reported that juiciness and flavor were significantly influenced by marbling level (slight, modest, slightly abundant, abundant).

Jennings et al. (1978) concluded that steaks from carcasses with modest or above marbling had higher (p<.05) tenderness and juiciness ratings and lower (p<.05) WBS force values than steaks containing slight or below marbling. In agreement with this, Dikeman et al. (1979) reported that WBS values were highest (p<05) for steaks practically devoid and traces marbling, and lowest (p<.05) for steaks from average modest or higher marbling scores. Additionally, Tuma et al. (1962) reported that WBS values were lower (p<.005) for steaks from slightly abundant than for slight marbled steaks.

In contrast to these findings, many researchers have reported that marbling level does not effect taste-panel tenderness, juiciness or flavor scores (Tuma et al., 1962; Goll et al., 1965; Field et al., 1966; Breidenstein et al., 1968; Norris et al., 1971; Parrish et al., 1973a; Garcia-de-Siles et al., 1977).

Furthermore, Breidenstein et al. (1968), Norris et al. (1971) and Jost et al. (1983) found no differences in WBS values due to marbling.

Marbling alone has been reported to account for little variation in meat tenderness. However, the interaction of marbling and maturity, which are combined for USDA quality grades, might have an effect on meat palatability. Skelley et al. (1976) reported that the acceptability of beef top-loin steaks showed very little dependence upon USDA quality grade. They showed that average Good (now Select) is a dividing point since steaks from carcasses grading high
Standard, low Good, or average Good were slightly inferior to steaks from carcasses grading high Good or higher. Simone et al. (1959) reported that taste panel tenderness, juiciness, and flavor scores were significantly higher for steaks from Choice grade than those from Good grade carcasses. Tatum et al. (1980) found steaks from high Choice and average Choice carcasses were significantly more juicy, flavorful and more desirable in overall palatability than steaks from low Good and High Standard carcasses. Similar results were reported by Dolezal et al. (1982b) who found steaks from Standard carcasses received the lowest (p<.05) ratings for all palatability attributes except juiciness, while steaks from Choice carcasses received the highest (p<.05) ratings for juiciness, flavor desirability, overall palatability, and had the lowest (p<.05) WBS force values. Smith et al. (1983) reported that taste panel flavor desirability of loin steaks decreased significantly between Prime, Choice, Good, and Standard grades.

In another study by Dolezal et al. (1982a) few differences existed in palatability between rib steaks from carcasses of different USDA quality grades. Additionally, other sensory evaluation studies indicated that quality grade did not significantly influence tenderness (Prost et al., 1975b; Skelley et al., 1976).

Berry and Leddy (1990) compared charbroiling and research type broiling on A-maturity strip loin steaks which varied in degree of marbling from moderately abundant to slight. They found no interaction between marbling level and cookery method and reported steaks with either moderately abundant or slightly abundant marbling were rated as more tender than steaks with moderate or modest, which were more tender than steaks exhibiting a small or slight degree of marbling. These results are similar to those reported by Smith
et al. (1984; 1987) and Savell et al., (1987). Furthermore, Parrish et al., (1991) reported rib and loin steaks from Prime were scored higher by trained sensory panelists than Choice, which were scored higher than Select for the palatability attributes of tenderness, juiciness, flavor intensity and desirability, and overall palatability. This disagrees with earlier work by Parrish et al. (1973a) who found no significant effect of quality grade on palatability of steaks.

Because marbling is such an important component of the USDA quality grading system beef, much research has been conducted on the various degrees of marbling and their effects on tenderness attributes. Marbling alone accounts for a very low percentage of the variation in tenderness. However, increased degree of marbling or quality grades may result in increased sensory panel scores and decreased WBS values.

Effect of Cookery on Muscle Proteins and Palatability

Meat cookery and the effect of heating on specific muscle components is very important, yet they have not been as thoroughly researched as many areas which have far less impact upon overall palatability. Several investigations, however, have been conducted on preslaughter management, nutrition, postmortem aging/handling, gender, physiological maturity, connective tissue characteristics, myofibrillar fragmentation, and proximate composition of muscle. As consumer preferences have moved towards reducing caloric intake and cutting down intakes of saturated fats, researchers have investigated research approaches to produce lean meat products of particular importance is the issue of how can cookery optimize desirable palatability attributes, especially those of leaner beef products.
**Effect of Cooking on Muscle Proteins and Flavor**

Nearly all meat is heated prior to consumption. Historically heating was for pasteurization effects, although applying heat produces characteristic flavor, aroma, texture and appearance attributes to which consumers have become accustomed. Hamm and Hofmann (1965) reported that characteristic flavors develop at temperatures exceeding 70°C when oxidation of -SH to -SS-groups from actomyosin occurs. Furthermore, during longer heating periods at higher temperatures, H₂S is formed from free or easily reacting groups of actomyosin.

Flavor changes during heating of meat are influenced by type of cookery and amount of heat applied to the system. Methods of cookery using dry heat, (broiling, roasting) exposes surface tissues to high temperatures which result in fluids progressively migrating to the surface. This acts to concentrate both water-soluble and lipid-soluble flavor components of the species on the surface (Paul, 1972). Furthermore, the texture of fat softens or liquifies. While some fat is lost, some coats the surface and forms aromatic compounds which are volatilized and provide materials for pyrolysis and/or interactions for all the components (Paul, 1972; Allen and Foegeding, 1981). Cookery methods utilizing moisture such as braising provide more optimal conditions for water-soluble flavor compounds to migrate from the meat into the broth or drippings (Paul, 1972).

Cooked meat flavor is also dependent upon internal temperature. Spanier et al., (1990) suggests hydrolytic enzymes such as lipases, glycosidases and proteinases undergo temperature induced changes which make them excellent effectors for the formation of meat flavor components. The proteinases show various activity levels at different cooking temperatures.
(Spanier et al., 1988) and can produce several reactive products, including flavor peptides (Yamasaki and Maekawa, 1978; Asao et al., 1987). Furthermore, proteinases are capable of generating a pool of amino acids for the formation of Maillard reaction products (Bailey, 1988). Spanier et al. (1990) reported the thiobarbituric acid reactive substance (TBA) level of beef semimembranosus muscles oven roasted at 177°C to 51.6, 60.0, 68.3 or 76.7°C was dependent on end point temperature. TBA levels increased with increasing internal temperature up to 68.3°C, above this temperature, TBA levels declined. Gas chromatography analysis revealed that hexanal increased to a maximum by 60°C and then steadily declined with increasing temperature. The drop in TBA and hexanal levels may be explained by an increase in the formation of Maillard reaction products.

Effect of Cookery on Meat Texture

In addition to characteristic flavors which evolve during heating, the texture of meat is also influenced to a large extent. In beef, tenderness may be the single most important palatability attribute determining consumer acceptance.

A large survey was recently conducted by researchers at Texas A&M University (Morgan et al., 1991) which suggested that slightly over 20% of the rib and loin cuts and over 40% of the round and chuck cuts would be rated "slightly tough" or tougher as based on Warner-Bratzler shear values related to sensory panel tenderness. Indeed, this is a problem. As one considers the genetic base of today's cattle, management/production systems, and packing/processing procedures, it is clear there are no simple solutions to
enhancing tenderness of beef cuts unless mechanical or enzyme induced tenderness systems are applied. However, these may be met with various concerns over food safety, purge, etc. Therefore, one key aspect of tenderness which seemingly has been overlooked is cookery. Can cookery optimize tenderness in certain cuts of beef that vary in connective tissue and fat composition?

Previous research by Davey and Gilbert (1974) and Bouton and Harris (1981) indicated that when meat is heated, two distinctly separate phases of toughening occur at different temperatures. In the first phase, a three to fourfold toughening occurs between 40°C and 50°C, believed to be due to the denaturation of the contractile system. The second phase occurs between 65°C and 70°C and results in a doubling toughness believed to be mediated by collagen shrinkage. In the 65°C to 70°C temperature range, the percentage fiber shrinkage and weight loss occurred. This also coincides with increased shear force also.

Several studies have correlated effect of final internal temperature to the tenderness of meat. Independent studies by Draudt (1972) and Parrish et al. (1973) have shown that degree of doneness had a greater influence on tenderness and overall palatability than did maturity or marbling. In fact, Parrish et al., (1973a) found no interaction with degree of marbling and internal cook temperature for steak palatability. They were first to report this. Furthermore, as internal temperature is increased, the meat becomes harder, drier and less tender as observed by sensory panel scores and shear force values (Cover et al., 1962; Ritchey and Hostetler, 1965; Parrish et al., 1973a; Hostetler et al., 1976; Cross et al., 1976; Bowers et al., 1987).
Effect of Heating on Juiciness and Water Holding Capacity

Juiciness is a commonly appreciated palatability attribute. It wets and refreshes the mouth and its absence violates expectations and lowers the perceived quality. High quality meats are expected to be juicy (Szczesniak, 1991). In a recent study, Szczesniak and Ilker, (1988) described juiciness as a multi-dimensional perception that includes force with which the juice squirts out, total amount of juice released on chewing, flow properties of the expressed liquid and the contrast in consistency between the liquid and suspended cell debris. Absence of juiciness means the product is dry and thus, will draw moisture from the mouth instead of supplying it to the mouth. Juiciness is a palatability attribute which may be closely linked to tenderness. It is influenced to a large extent by the degree of doneness.

Laakonen et al. (1970a) reported that degree of doneness in meat is extremely critical in affecting tenderness and cooking yield. They found if the final internal temperature is below the collagen shrinkage temperature (60°-65°C), then the major reduction in tenderness does not occur. Conversely if final temperature is greater than that of the collagen shrinkage temperature, the coagulation results in a higher cook loss percentage and more tightly packed, less tender muscle, (Laakonen et al., 1970b). Several researchers have reported as final internal temperature increases, juiciness decreases (Ritchey and Hostetler, 1965; Parrish et al., 1973a; Cross et al., 1976; Bowers et al., 1987).

Cooking meat causes protein denaturation followed by coagulation of myofibrillar proteins, shrinkage of myofilaments and a tightening of the microstructure of myofibrils (Cheng and Parrish, 1979). Since lean cuts of fresh
beef contain approximately 75 percent water with protein being the principle component responsible for binding that water, cookery plays an important role in maintaining maximum cooking yield. According to Davey and Gilbert (1974), the shrinkage of collagen caused by heating above 60°C caused a contracture of the collagen sheath and an exudation of moisture from the myofibrils.

When considering the meat's inherent ability to bind water, only 4-5 percent is tightly bound to actin or myosin, depending on the shape or charge of the protein (Hamm, 1969). Since this represents a small fraction of total water, it is of less importance when compared to the fraction immobilized within the muscle tissues which is influenced by the spacial molecular arrangement of myofibrillar proteins (Hamm, 1969). Tightening the spacial network of myofibrillar proteins by heating decreases amount of immobilized water. Therefore, amount of expressible moisture increases (Hamm, 1969; Hostetler and Landmann, 1968).

It appears that moisture loss in cooked meat contributes to decreased tenderness. Although this coincides with events related to myofibrillar denaturation and collagen shrinkage, juiciness and tenderness are closely related.

Sensory Evaluation

Sensory evaluation has been defined as a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of food
and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (IFT, 1975).

There are many common industrial applications in which sensory evaluation is useful. These might include new product development, product matching, product improvement, process change, cost reduction and/or selection of a new source supply, quality control, storage stability and product grading or rating (IFT, 1981).

When considering meat products, and specifically, beef, many of these criteria could apply. For example, ratings based on carcass quality grades or evaluations of differences in postmortem or antemortem treatments applied to carcasses and/or meat or live animals, respectively, are common reasons to conduct sensory evaluation research to determine if, and to what extent, treatment variables influence palatability attributes.

In 1978, the American Meat Science Association published helpful guidelines to conduct fresh meat sensory evaluation in order to improve consistency among various institutions that are routinely involved with meat palatability investigations (AMSA, 1978). These guidelines have served their purpose well. However, a committee has recently been appointed to update these guidelines to allow greater flexibility and creativity in panel training, product preparation, presentation and scoring systems.

Most fresh beef sensory panel research investigations have chosen to report specific palatability attributes using a hedonic scale. This is used to reflect the respondents' perceived intensity of a specified attribute under a given set of conditions. This test is used to measure the liking for food products. The method relies on test subjects' capacities to report directly and indirectly, their
feelings of like and dislike. Several variations of the traditional nine-point word hedonic scale have been used effectively. These include: 1) a reduced number of rating categories; 2) omission of the neutral category; 3) substitution of verbal categories by caricatures representing degrees of pleasure and displeasure such as a facial hedonic scale; 4) use of a non-structured, non-numerical line scale anchored with "like" and "dislike" on opposite ends (IFT, 1981).

Hedonic scale ratings are converted to numerical scores, and statistical analysis is applied to determine differences in degree liking between or among samples. A hedonic rating test can yield both absolute and relative information about test samples. Absolute information is derived from degree liking or disliking indicated for each sample, and relative information is gained from direction and degree of difference between or among the sample scores (IFT, 1981).

In order to conduct sensory panel investigations effectively, thought must be given to panelist selection and training (AMSA, 1978). Persons should be willing, enthused, have at least average sensitivity and be in good health (Larmond, 1970). After an initial panel callout, trainings sessions led by an experienced person must be conducted. These training sessions are intended to familiarize potential panelists with the attributes each will score, the sensory panel form, and product which varies considerably in intensity from degree of "like" to "dislike." Panelists are then evaluated on ability to be consistent within the same session and between sessions. This is generally done by statistical methods using a standard error of repeatability as a measure (AMSA, 1978).
Once a panel has been trained and selected, consideration must be given to the physical surroundings in which the panels will be conducted. Control of lighting, temperature, aroma and individual partitions are necessary to allow an individual panelist the greatest opportunity to concentrate on the task at hand.

Since trained sensory panelists are important in the data gathering process, they must be rewarded in some manner for their contributions. It is imperative that sensory panels start promptly, finish in a timely fashion, and that each panelist gains personal satisfaction from their participation.

Mechanical Measures of Meat Tenderness

In addition to subjective assessment of meat palatability by sensory panelists, instrumentation has been developed to objectively characterize certain palatability attributes. Various types of instruments which include penetrometers, masicometers, extruders and shear devices have been used to determine meat tenderness attributes.

Penetrometers are based on the principle of inserting a probe or needle into the tissue and measuring the force required for a given penetration depth. These systems have a strain-guage associated with each probe and the force reading is dependant upon the resistance of the tissue. The Armour Tenderometer is such a device. It was hoped that this system could accurately segregate beef carcasses into tenderness groups and thus carcasses be marketed accordingly. However, it was not shown to be a reliable indicator of tenderness
Various masicometers, extruders and cutting devices have been used to determine tenderness of muscle and meat (Szczesniak and Torgeson, 1965; Szczesniak, 1973). These have not been extensively used in meat science textural studies, particularly when compared with devices that measure shear force.

The Warner-Bratzler (WB) shear device is the best known apparatus which meat scientists use to objectively and quantitatively assess meat tenderness. This device is composed of a 1mm thick metal blade which has a triangular opening into which a cylindrical meat sample core, usually either 1.27 or 2.54 cm in diameter, is inserted (Bratzler, 1949). Shear bars on each side of the triangular blade are driven downward through the longitudinal axis of the muscle fibers in the core which separates it into two pieces. The maximum force exerted on the blade is detected by a dynameter spring and read from a dial scale or from a printout if using a WB test cell fitted for the Instron Universal Testing Machine.

Bouton et al. (1975) and (Seideman and Theer, 1986) investigated shear force value and certain relationships associated with tenderness. When Bouton et al. (1975) analyzed shear force deformation curves, they found that the curves contained two segments. They reported that the first curve, initial yield force required to compress and initiate shear fracture planes through myofibrils, was mainly influenced by myofibril strength. The second portion of the curve, the difference between the initial yield force and the peak force, could be an indication of the strength of the connective tissue. Furthermore,
these investigators found that initial yield force values: 1) increased with internal temperature as degree of doneness exceeded 60°C; 2) decreased with advancing postmortem storage interval; 3) was not significantly influenced by animal age. In addition, Bouton et al. (1975) reported the difference between initial yield and peak force was: 1) not influenced by postmortem aging of carcasses from young or old animals; 2) significantly increased with animal age; 3) significantly reduced by cooking meat to a 90°C internal temperature.

It has been shown that high correlations between sensory panel tenderness and WB shear values exist. Previously reported correlations reported between sensory panel tenderness scores and WB shear values include: \( r = 0.62 \) Huffman, 1974; Olson and Parrish, 1977; Crouse et al., 1978); \( r = -0.63 \) (Goll et al., 1965); \( r = -0.65 \) (Moe et al., 1964); \( r = -0.75 \) (Field et al., 1966); \( r = -0.78 \) (McBee and Wiles, 1967); \( r = -0.88 \) (MacBride and Parrish, 1977); and \( r = -0.90 \) (Culler et al., 1978). Certain studies, however, have found poor correlations between sensory panel tenderness and WB shear value. Breidenstein et al. (1968) reported a correlation of \( r = -0.33 \) and Parrish et al. (1973b) reported a correlation of \( r = -0.30 \) for sensory panel tenderness and WB shear value.

Part of the reason for variation observed between WB shear value and sensory panel tenderness may be due to lack of homogeneity of the meat tissue. Shear tests have been used by many researchers who have evaluated core position effect in beef muscles. Alsmeyer et al. (1965) and Tuma et al. (1962b) found greater tenderness in the medial region of the beef longissimus. Furthermore, cores from the dorsal region were more tender than those from the central or lateral region in longissimus muscle roasts (Williams et al., 1983).
Smith et al. (1969) found the greatest tenderness in beef steak was in the central region whereas, Sharrah et al. (1965) and Cover et al. (1962) reported that the lateral region was the most tender. These last two studies agree with Crouse and coworkers (1989), who reported tenderness of cores improved from dorsal to lateral. Therefore, location effects of tenderness within the longissimus muscle could be useful in future studies of the physical/biochemical effectors of tenderness.

Various research institutions, in attempts to minimize expenses (time and financial), have elected to simply obtain WB shear values and dispense with sensory panel research. However, tenderness is the most important palatability attribute. Since near perfect correlations between sensory panel tenderness and WB shear values do not exist, and experienced meat sensory researchers agree that if discrepancies exist between sensory panel and WB shear values, then the WB value is the less reflective observation when compared with sensory panel result. Therefore, WB shear values should complement sensory panel research and not be used as a sole reflection of palatability (personal communication: Dikeman, 1987; Parrish, 1993).

Chemical Measures of Meat Tenderness

Research conducted principally by investigators from Iowa State University have found that degradation of the Z-line was related to the reduction of fiber tensile strength which is correlated with meat tenderness. Parrish et al. (1973c) found that smaller myofibril fragments originated from the more tender meat samples. Over the next several years, this group showed that
myofibrils fragmented as postmortem storage length advanced, fragmentation varied with different muscles and different storage temperatures, and extent of myofibrillar fragmentation could be quantified (Olson et al., 1976).

Furthermore, as these investigators began studying proteolytic and ultrastructural relationships involved with meat tenderness, it was discovered that the disappearance of troponin T and increased intensity of a 30 K dalton component during advanced postmortem storage was correlated with increased sensory panel tenderness, increased myofibril fragmentation index and reduced WB shear force (Olson and Parrish, 1977). Subsequent studies have shown that calcium dependent protease was responsible for this myofibrillar protein degradation.

MacBride and Parrish (1977) reported myofibril fragmentation index is perhaps the most important state of tenderness from meat in conventionally aged beef carcasses. Further investigations have confirmed the relationship between myofibril fragmentation index and beef tenderness (Olson and Parrish, 197; Culler et al., 1978).

A recent investigation designed to predict beef tenderness at day 14 postmortem combined mechanical and chemical procedures early postmortem. These investigators incorporated WB shear values, myofibrillar fragmentation index procedures and also determined calcium dependent protease inhibitor activity from samples at time of slaughter, again at 24 hours and 24 hour calpastatin activity. They found that when combined in a model, these three biochemical markers explained 63 percent of the variation in 14 day WB shear values of beef longissimus muscle (Shackelford et al., 1991).
Presently biochemical research designed to evaluate calcium dependent protease inhibitor activity combined with evaluating effects on ultrastructure and meat tenderness is being conducted by various groups in the United States and abroad. Further enhancing our understanding of fundamental mechanisms associated with tenderness will be of benefit to consumers and all parties involved with the production of livestock and its conversion to meat.
PAPER I. EFFECTS OF BROILING TEMPERATURE AND DEGREE OF DONENESS ON PALATABILITY OF BEEF STRIP LOIN STEAK FROM DIFFERENT USDA QUALITY GRADES
Effects of broiling temperature and degree of doneness on palatability of beef strip loin steak from different USDA quality grades

Johnson, R.D. and F.C. Parrish, Jr.
Department of Animal Science
Iowa State University, Ames

Running title: Effects of broiling on steak palatability

Journal paper no J of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No.
ABSTRACT

The objective of this study was to determine the effect of broiling temperature and degree of doneness on palatability attributes of beef loin strip steaks differing in quality grade. Seven strip loins were obtained from each of USDA Standard, low Select, high Select, low Choice, and average to high Choice. These 35 strip loins were vacuum packaged, stored 9 days postmortem (PM) at 0 to 2°C, cut into 3.0 cm thick steaks, frozen, and stored at -20°C. In addition, seven USDA low to average Prime strip loins were stored 23 days before cutting into individual steaks, vacuum packaged, frozen, and stored at -20°C. Steaks were thawed overnight to 3°C and broiled either at 246°C or 316°C to an internal temperature of either 62.8°C (medium-rare) or 70°C (medium). Steaks were served warm to a 10-member trained sensory panel for determination of tenderness, juiciness, flavor intensity, and overall palatability. Panelists scored steaks using an eight-point descriptive scale. Adjacent steaks were used for determination of Warner-Bratzler shear force (WBS) and cooking yield. Steaks broiled to 62.8°C had higher (p<.01) scores for tenderness, juiciness, flavor, and overall palatability and lower (p<.05) WBS values than those heated to 70°C. Steaks cooked in the 316°C broiler and those cooked to the higher degree of doneness were scored less juicy (p<.05) than those cooked in the 246°C broiler or to a lesser degree of doneness. Few palatability differences were found in steaks from either Choice or Select quality grades. Steaks from the Standard quality grade, however, were scored lower for palatability (p<.05) than steaks from other treatments, and steaks from the Prime quality grade scored higher for palatability (p<.05) than steaks from other treatments. Degree
of doneness (medium-rare) of steaks with a slight degree of marbling or above was of greater importance than broiler temperature or quality grade.
INTRODUCTION

USDA quality grade standards were formulated in 1926. Although several revisions have been made since the adoption of the 1926 Official United States Standards for Grades of Carcass Beef, degree of marbling and bone maturity, along with color and texture of lean, are very similar to the original standards. Quality grade standards are used extensively and have a major impact in the marketplace in establishing price. Recently, a survey study was undertaken to evaluate consumer acceptance of lean beef compared with traditional Choice beef. The results suggested that regional differences may exist and that certain consumers preferred more highly marbled beef, whereas others preferred leaner beef, and that consumers did not want to pay for excess trimmable fat (Savell et al., 1987). In response to concerns of merchandising leaner beef, the USDA quality grade Good was renamed USDA Select. Since this change in 1987, a greater percentage of cattle have been graded USDA Select by federal meat graders.

Parrish (1981) and Dikeman (1987) provide comprehensive reviews on the relationship of marbling to beef steak palatability attributes. Many investigations have found that rib or loin steaks from USDA Standard and low Select carcasses scored lower for palatability and/or shear force value than Choice, and these were scored lower than Prime (Tatum et al., 1980; Dolezal et al., 1982; Smith et al., 1984; Savell et al., 1987; Smith et al., 1987; Berry and Leddy, 1990; Parrish et al., 1991). However, other studies would indicate marbling would have a minimal influence on beef steak palatability or WBS values (Parrish et al., 1973; Armbruster et al., 1983; Jost et al., 1983).
Variations in degree of doneness have been shown to influence beef palatability attributes (especially tenderness) and WBS values. Investigations by Davey and Gilbert (1974) and Bouton and Harris (1981) indicated that when beef is heated, two distinctly separate phases of toughening occur at different temperatures. In the first phase, a three to fourfold toughening occurs between 40°C and 50°C, believed to be due to the denaturation of the contractile system. The second phase occurs between 65°C and 70°C and results in a doubling of toughness believed to be mediated by collagen shrinkage. In the 65°C and 70°C temperature range, the largest incremental percentage of fiber shrinkage and weight loss occurred which coincided with increased shear force.

Several studies have evaluated the effect of final internal temperature to the tenderness of beef. Draudt (1972) and Parrish et al. (1973) have shown that degree of doneness had a greater influence on tenderness and overall palatability than did maturity or marbling. In fact, Parrish et al. (1973) found no interaction with degree of marbling and internal cook temperature for steak palatability. Investigations have shown that as internal temperature is increased, meat becomes harder, drier, and less tender as observed by sensory panel scores and shear force values (Cover et al., 1962; Ritchey and Hostetler, 1965; Parrish et al., 1973; Hostetler et al., 1976; Cross et al., 1976; Bowers et al., 1987).

Although the AMSA (American Meat Science Association) meat cookery guidelines have provided for improved standardization of cookery/sensory methods among research institutions, recommendations of cooking beef steak to a 70°C degree of doneness in light of known temperature-toughness relationships (Davey and Gilbert, 1974; Bouton and Harris, 1981) seems ill
advised, particularly with results of the National Beef Tenderness Survey which indicated approximately 20 percent of rib and loin steaks and 40 percent round and chuck cuts were outside the acceptable range for tenderness (Morgan et al., 1991).

The objective of this study was to determine the relationship of different degrees of marbling (USDA quality grade) to cooked beef palatability as influenced by broiler temperature and degree of doneness.
MATERIALS AND METHODS

Selection of Strip Loins and Processing of Steaks. Cattle (steers) used in this study were genetically similar (1/2 Angus, 1/4 Hereford, 1/8 Jersey, 1/8 Simmental), and were fed a high concentrate corn-based finishing ration at the Rhodes Experiment Station, Rhodes, IA. Cattle were slaughtered at an average of 14.5 mo of age (USDA Standard-Average to High Choice) at a commercial packing plant, or at an average of 18.5 mo of age (USDA Prime) at the Iowa State University Meat Laboratory.

USDA yield and quality grades were determined 24 hr postmortem by three trained personnel from Iowa State University and one USDA Grader. Seven strip loins (I.M.P.S. 180) were removed 48 hr PM from each of the following USDA quality grade carcasses: Standard, low Select, high Select, low Choice and average to high Choice. These 35 strip loins were vacuum-packaged, stored 9d at 0°C to 2°C, cut into 3.0 cm thick steaks, frozen and stored at -20°C. The remaining seven Prime strip loins were stored for 23d at 0°C to 2°C, cut into individual steaks, vacuum-packaged, frozen and stored at -20°C. Steaks were thawed 26 hr at 3°C before broiling and subcutaneous fat was trimmed to a maximum thickness of 0.4 cm.

Immediately after removal of each strip loin, an anterior slice was removed from each. Slices were carefully trimmed of all subcutaneous fat, epimysium and peripheral muscles so that only the completely trimmed longissimus muscle remained. Each slice was frozen with the use of liquid nitrogen and powdered in a Waring Blender. The pulverized muscle was stored in Whirl-Pak bags at -20°C until laboratory determinations were made.
Lean from each strip loin steak was analyzed for percentage extractable lipid and percentage moisture following AOAC (1990) procedures. The longissimus muscle samples (about 3.5 gm) were loaded into oven-dried cotton thimbles, weighed and placed in a drying oven for at least 24 hr. Upon removal from the oven, all samples were reweighed (for determination of moisture loss) and were subjected to lipid extraction using Soxhlet apparati and n-hexane as the solvent. After a 7 hr extraction period, samples were removed, air dried to remove most of the n-hexane, and placed in the drying oven for at least 24 hr before reweighing (for determination of lipid loss). All determinations were made in triplicate and only those that were within 5% of each other after computation to determine moisture and lipid content were accepted (all others were reanalyzed until acceptable figures were obtained).

**Broiling Procedures.** Two broiling procedures were used in this study. The first method involved using an electric consumer household oven broiler. Specifically, steaks were placed on broiler pans and cooked on the top rack where the meat surface was 10 cm from the heat source. Steaks were broiled at 246°C ± 14°C and first turned when the internal temperature reached 39°C. Internal temperatures were monitored with iron constantan thermocouples inserted into the geometric center of each steak and attached to an Omega Digital Trendicator. Steaks were removed from this broiler at either 59°C or 65°C to account for post cooking temperature rise to achieve a final degree of doneness of either 62.8°C or 70°C.

The second method involved broiling steaks using procedures typical to restaurant situations. Steaks were broiled in a large stainless steel electric broiler at 316°C ± 8°C. They were placed directly on the broiler rack, turned at 39°C and removed when the internal temperature reached 57°C or 63°C to
achieve a final degree of doneness of 62.8°C or 70°C respectively as monitored by use of thermocouples.

After determining final internal temperatures, steaks were wrapped in aluminum foil and placed in an oven preheated to 60°C prior to cutting into samples for serving to trained sensory panelists.

Sensory Evaluation. Steaks were evaluated by a ten member sensory evaluation panel trained according to the American Meat Science Association (AMSA, 1978). Steaks were cut into 1.25 cm x 1.25 cm x thickness of steak cubes and evaluated for tenderness, juiciness, flavor intensity, flavor desirability and overall palatability. Eight-point structured scales were used where 8 = extremely tender, juicy, intense beefy flavor, desirable flavor and palatable whereas, 1 = extremely tough, dry, bland, and unpalatable. Also, steaks weights were recorded before and after cooking to determine cooking loss.

Panelists were seated in individually partitioned booths with red fluorescent overhead lighting to mask differences due to degree of doneness. Panelists were provided water (ambient temperature) along with unsalted crackers and "Granny Smith" apple slices to cleanse their palates between samples. One warm-up sample was followed by eight samples for sensory evaluation at each panel session.

Four steaks from a given strip loin were all evaluated in the same session to negate variation due to strip loin and determine with greater confidence, the influence of degree of doneness and broiler method. Two strip loins (four steaks each) from each marbling group were randomly selected from the 15 possible quality grade combinations and samples from these steaks were presented to panelists at each session. In total, 28 steaks from each marbling degree treatment were served to panelists; 14 at the low degree of doneness
and/or prepared by household oven broiling methods, and 14 at the higher
degree of doneness and/or prepared in a commercial restaurant broiler.
Sensory panelists scored palatability attributes on a total of 168 strip loin steaks.

Instron Shear Values. Four additional steaks per strip loin adjacent to
those evaluated by sensory panelists were used for WBS determination. After
broiling, steaks were allowed to cool to 25°C and seven 1.26 cm uniformly
distributed cores were removed with a mechanical coring device perpendicular
to the surface for each steak. Cores were sheared twice and values averaged
using a Warner-Bratzler test cell apparatus fitted for a Instron Universal
Testing Machine (Instron Corporation, Canton MA). A 50-Kg load cell and a
cross-head speed of 200 mm/min was used. Peak shear force is expressed in Kg.

Statistical Analyses. Analysis of variance procedures (SAS, 1990) were
used on the data and included marbling group, degree of doneness and broiler
method as main effects and marbling group x degree of doneness, marbling
group x broiler method, degree of doneness x broiler method and marbling
group x degree of doneness x broiler method interaction. None of the
interactions were significant. Therefore, means for main effects will be
reported. Fisher's least significant difference procedures were used for
separation of the means.
RESULTS AND DISCUSSION

The quantitative and qualitative carcass characteristics grouped according to quality grade are presented in Table 1. Hot carcass weights according to quality grade were similar (p>.05). Fat thickness was greater (p<.01) for carcasses grading Prime. Ribeyes areas were smaller (p<.05) in carcasses grading low Choice. Prime carcasses had the highest (p<.05) percentage of kidney knob and numerical yield grade. Marbling scores increased (p<.01) in a step-wise progression from Standard through Prime grades, although low Select and high Select grades were similar (p>.05). Longissimus muscle lipid content increased (p<.05) from Standard through Prime with the exceptions of low and high Select and low Choice grades (p>.05).

Palatability attributes affected solely by the main effects of quality grade, degree of doneness and broiler temperature (no interaction, p>.05) are shown in Tables 2, 3 and 4, respectively. Steaks from Standard grade carcasses scored lowest (p<.05) for tenderness and were numerically lowest for all sensory attributes evaluated (Table 2). In contrast, steaks from Prime carcasses scored highest (p<.05) for tenderness, flavor intensity and overall palatability and were numerically highest for juiciness. Additionally, WBS values were in close agreement with values obtained by sensory panelists (Figure 1). These results as influenced by quality grade, are similar to those recently reported by Smith et al. (1984), Savell et al. (1987), Berry and Leddy (1990) and Parrish et al. (1991) for strip loin steaks within A maturity carcasses. These investigators reported that steaks from Prime were scored highest and as quality grade decreased palatability was reduced. However, in this study, steaks from young Select and
Choice cattle (14.5 mo of age) were scored similarly. In addition, Prime steaks in this study were stored 23d compared with 9d for steaks from all other quality grades. This would likely improve the tenderness of the Prime quality grade steaks used in this study. This would be in agreement with Huff and Parrish (1993) who reported strip loin steaks improved significantly in tenderness from day 7 to day 28.

Degree of doneness had a significant influence on sensory properties (Table 3). Steaks broiled to an internal temperature of 62.8°C scored higher (p<.05) for tenderness, juiciness, flavor intensity and overall palatability than those broiled to 70°C. Furthermore, WBS values support sensory panel tenderness ratings (Figure 2). In addition, percentage cooking loss was lower (p<.05) for steaks broiled to 62.8°C (Figure 3) This difference (6%) supports sensory panel juiciness ratings. This compares favorably with Parrish et al. (1973) who first reported that degree of doneness may have a more important relationship to palatability of steaks than marbling in A maturity beef carcasses and found no interaction between degree of doneness and degree of marbling. Furthermore, when considering effects of heating on muscle proteins and moisture retention, Laakonen (1970a,b) found that if the internal temperature exceeded 65°C to 70°C, the protein coagulation resulted in a more tightly packed, less tender muscle with a higher cook loss percentage. Conversely, if the final internal temperature was below the collagen shrinkage temperature (60° to 65°C), then major reduction in tenderness did not occur. Smith and Carpenter (1974) reported that steaks containing higher degrees of marbling provide "insurance" in maintaining tenderness when meat is cooked at high temperatures to well-done internal temperatures. Because we cooked steaks to
a medium-rare or medium degree of doneness, we cannot assess whether sensory properties would be more favorable for steaks from higher quality grades cooked to a well-done endpoint. However, optimal palatability based on our results, regardless of quality grade, seems to be achieved by cooking strip loin steaks to an internal temperature of doneness of 62.8°C.

Broiling temperature influenced the juiciness score of steaks (Table 4). Those steaks broiled at 246°C in the household oven-broiler were juicier (p<.05) than steaks broiled at 316°C in the commercial restaurant broiler. No other palatability differences were influenced by broiler temperature (p>.05). Furthermore, WBS values were similar (p>.05) regardless of broiling temperature (Figure 4). Cooking loss percentage was higher (p<.05) for steaks broiled at 316°C compared with 246°C (Figure 5). The magnitude of this difference was about 6 percent, and it is likely that sensory panelists were able to detect this. This agrees with Cross et al., (1976) who found differences in initial or sustained juiciness due to higher oven temperatures (177°C or 232°C) in longissimus muscle steaks. However, unlike our results, Cross et al., (1976) found sensory panel tenderness scores were reduced when steaks were roasted to 60°C or 70°C with use of high oven temperatures. In addition, Cross et al., (1979) reported that at the same degree of doneness, steaks roasted in an oven (175°C) were more tender than those cooked in an electric broiler (275°C). Recently, Berry and Leddy (1990) found slightly higher tenderness scores resulted from charbroiling (300°C) compared with electric broiling (225°C).
Table 1. Quantitative and qualitative carcass characteristics for USDA quality grades

<p>| USDA Quality Grade | Carcass Characteristics | | | | | |
|--------------------|-------------------------|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th></th>
<th>Hot Carcass Wt. (Kg)</th>
<th>Fat Thickness (cm)</th>
<th>Ribeye Area (cm²)</th>
<th>Kidney Knob (%)</th>
<th>U.S.D.A. YG</th>
<th>Marbling Score</th>
<th>Longissimus M. Lipid Content % (Lean Only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (n=7)</td>
<td>337</td>
<td>0.6f</td>
<td>86.5a</td>
<td>2.3a</td>
<td>2.1a</td>
<td>Tr49f</td>
<td>2.2a</td>
</tr>
<tr>
<td>Low Select (n=7)</td>
<td>339</td>
<td>0.9f</td>
<td>87.4a</td>
<td>2.7ab</td>
<td>2.4ab</td>
<td>Sq27g</td>
<td>3.4b</td>
</tr>
<tr>
<td>High Select (n=7)</td>
<td>349</td>
<td>0.8f</td>
<td>85.8a</td>
<td>2.4a</td>
<td>2.5ab</td>
<td>Sq69g,h</td>
<td>4.1,b,c</td>
</tr>
<tr>
<td>Low Choice (n=7)</td>
<td>314</td>
<td>0.8f</td>
<td>75.5b</td>
<td>2.7a,b</td>
<td>2.3a,b</td>
<td>Sm44i</td>
<td>5.4c</td>
</tr>
<tr>
<td>Ave-High Choice (n=7)</td>
<td>310</td>
<td>0.9f</td>
<td>79.4ab</td>
<td>2.5ab</td>
<td>2.6b</td>
<td>Md09j</td>
<td>7.2d</td>
</tr>
<tr>
<td>Prime (n=7)</td>
<td>344</td>
<td>2.0g</td>
<td>78.1ab</td>
<td>2.9b</td>
<td>4.1c</td>
<td>Slab34k</td>
<td>10.5e</td>
</tr>
<tr>
<td>SE</td>
<td>1.8</td>
<td>0.1</td>
<td>3.2</td>
<td>0.2</td>
<td>0.2</td>
<td>17</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a,b,c,d,e Means within a column with different superscripts differ (P<.05).

Table 2. Effect of USDA quality grade on palatability attributes of beef strip loin steaks broiled at different temperatures and to different degrees of doneness

<table>
<thead>
<tr>
<th>USDA Quality Grade</th>
<th>Sensory Attribute</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tenderness</td>
<td>Juiciness</td>
<td>Flavor Intensity</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Standard (n=28)</td>
<td>5.5a</td>
<td>5.4a</td>
<td>5.4a</td>
</tr>
<tr>
<td>Low Select (n=28)</td>
<td>6.3b,c</td>
<td>5.7a,b</td>
<td>5.6a,b</td>
</tr>
<tr>
<td>High Select (n=28)</td>
<td>6.0b,c</td>
<td>5.8a,b</td>
<td>5.7b</td>
</tr>
<tr>
<td>Low Choice (n=28)</td>
<td>6.4c</td>
<td>6.0b,c</td>
<td>5.8b</td>
</tr>
<tr>
<td>Ave-High Choice (n=28)</td>
<td>6.1b,c</td>
<td>5.9b,c</td>
<td>5.7b</td>
</tr>
<tr>
<td>Prime (n=28)</td>
<td>7.0d</td>
<td>6.2c</td>
<td>6.5c</td>
</tr>
<tr>
<td>SE</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

a,b,c,d Means within a column with different subscripts differ (P<.05).
Table 3. Effect of degree of doneness on palatability attributes of beef strip loin steaks from all USDA quality grades broiled at different temperatures

<table>
<thead>
<tr>
<th>Internal Temperature</th>
<th>Sensory Attributes</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tenderness</td>
<td>Juiciness</td>
<td>Flavor Intensity</td>
<td>Overall Palatability</td>
</tr>
<tr>
<td>62.8°C (n=84)</td>
<td>6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>70.0°C (n=84)</td>
<td>6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> means within a column with different superscripts differ (P<.05).

Table 4. Effect of broiling temperature on palatability attributes of beef strip loin steaks from all USDA grades cooked to different internal temperatures

<table>
<thead>
<tr>
<th>Broiling Temperature</th>
<th>Sensory Attributes</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tenderness</td>
<td>Juiciness</td>
<td>Flavor Intensity</td>
<td>Overall Palatability</td>
</tr>
<tr>
<td>246°C (n=84)</td>
<td>6.3</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>316°C (n=84)</td>
<td>6.1</td>
<td>5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7</td>
<td>5.7</td>
</tr>
<tr>
<td>SE</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means within a column with different superscripts differ (P<.05).
Figure 1. Effect of USDA quality grade on WBS values of beef strip loin steaks broiled at different temperatures to different degrees of doneness

Quality Grade

a, b, c Means with different superscripts differ (p<0.05).
x Each bar is the mean of 28 observations.
Figure 2. Effect of degree of doneness on WBS of beef strip loin steaks from different USDA grades broiled at different temperatures

a,b Means with different superscripts differ (p<.05)
Figure 3. Effect of degree of doneness on weight loss of beef strip loin steaks from different USDA quality grades broiled at different temperatures.

Means with different superscripts differ (p<.01).
Figure 4. Effect of broiling temperature on WBS of beef strip loin steaks from different USDA quality grades cooked to different internal temperatures
Figure 5. Effect of broiler temperature on weight loss of beef strip loin steaks from different USDA quality grades cooked to different internal temperatures.

a,b Means with different superscripts differ (p<.01).
IMPLICATIONS

As preferences toward consumption of lean beef increase, methods to optimize palatability must be explored, particularly with respect to cookery. Degree of doneness (medium-rare) of steaks with at least a slight degree of marbling was of greater importance than broiler temperature or quality grade. This may be important for retail cuts from young calf-fed beef, typically having a slight or small degree of marbling, for ensuring desirable palatability.
REFERENCES CITED


PAPER II. EFFECTS OF QUALITY GRADE, BROILER TEMPERATURE AND DEGREE OF DONENESS ON CHOLESTEROL CONTENT FATTY ACID AND PROXIMATE COMPOSITION AND CALORIC CONTENT OF BEEF STRIP LOIN STEAKS
Effects of quality grade, broiler temperature and degree of doneness on cholesterol content, fatty acid and proximate composition and caloric content of beef strip loin steaks

Johnson R.D., F.C. Parrish, Jr. and S.L. Nissen
Department of Animal Science
Iowa State University, Ames

Running title: Chemical composition of beef loin steaks

Journal paper no J. of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No.
ABSTRACT

The objectives of this investigation were to develop an accurate and rapid method to determine cholesterol content (CC) of lean from raw and cooked beef strip loin steaks which differed in quality grade and subcutaneous fat (SQ) trim level. Seven strip loins were removed from each of USDA Standard, low Select, high Select, low Choice, average to high Choice and low to average Prime carcasses. The 42 strip loins were vacuum-packaged, stored 9 d at 0°C to 2°C (except Prime = 23 d), cut into 3.0 cm thick steaks trimmed to 0.4 cm of SQ fat and frozen and stored at -20°C until analyzed. After thawing, steaks were broiled at either 246°C or 316°C to an internal temperature of 62.8°C or 70°C.

Determination of CC on liquid nitrogen pulverized tissue was accomplished by a rapid direct transesterification procedure followed by gas chromatography-mass spectrometry techniques (GCMS). Raw Prime steaks had a higher (p<.05) CC than those from Standard and Select. However, no differences (p>.05) were observed among quality grades after broiling.

Although significant differences in CC were found in raw steaks, the magnitude of these differences was too small to be of practical importance. Of importance, however, lower CC reported in this paper relative to most previously published reports are likely due to improved accuracy and precision of using GCMS techniques. These techniques are specific for cholesterol.

Determination of fatty acids was accomplished by using a direct transesterification technique followed by gas chromatographic analysis. We found a lower percentage (p<.05) of C16:0 and higher percentage (p<.05) of C18:2
in lean from raw Standard grade steaks than Select, Choice or Prime. Lean from broiled, Standard and low Select steaks had a higher percentage (p<.05) of C18:0 and C18:2 and lower percentage (p<.05) of C18:1 compared with high Select, Choice and Prime. Across all quality grades, steaks broiled to 62.8°C had a higher percent (p<.05) of C18:0 than those broiled to 70°C. Steaks broiled at 246°C had a higher percent (p<.05) of C14:0 and C18:2 and lower percent (p<.05) of C18:1 than those broiled at 316°C.

For both raw and cooked strip loin steaks, as quality grade increased lipid content (LC) and caloric density (CD) increased (p<.05) and moisture content (MC) decreased (p<.05). In lean from broiled steaks only, ave-high Choice and Prime had a lower (p<.05) percentage of protein (PC) than Choice and Select and these were higher than Standard (p<.05). Steaks broiled to 62.8°C or those broiled at 246°C had a lower (p<.05) LC and a higher (p<.05) MC than those cooked to 70°C or broiled at 316°C. Steaks broiled to 70°C had a higher (p<.05) than those broiled to 62.8°C.

Small, yet significant differences in fatty acid composition were found, the magnitude of the differences may be too low to be of practical importance. However, as quality grade increases, LC in cooked lean and calories from fat increases. Also, eliminating external SQ fat prior to cooking steaks may have little impact on LC or CD.
INTRODUCTION

Consumers have been advised by many scientists, nutritionists and clinicians to eat foods with less cholesterol and fat to decrease risk of coronary heart disease. This recommendation is based on evidence that a decrease in fat intake, especially saturated fat, will decrease serum cholesterol concentration. Also, decreased fat intake may decrease mortality from a variety of cancers (CAST, 1991).

Several researchers have analyzed cholesterol content of beef which differed in quality grade. Stromer et al. (1966) found that longissimus muscle contained 36-46 mg/100 gm tissue. Recently, Rhee et al. (1982a) reported 52-66 mg/100 gm (raw) and 76-93 mg/100 gm (cooked to 60° or 75°C) for strip loin steaks differing in marbling from practically devoid to moderately abundant. Conversely, Berg et al. (1985) found no consistent trends for cholesterol from rib steaks ranging from practically devoid to moderate when reported on a dry matter basis. Hoelscher et al. (1988) observed no difference for raw Select, Choice or Prime strip loin steaks; however, cooked Prime steaks were higher in cholesterol than were cooked Choice or Select steaks. Increasing final internal temperature was shown to increase CC (Rhee et al., 1982a).

Fatty acid composition of beef does influence properties associated with flavor (Hornstein, 1971; Pearson et al., 1977; Melton et al., 1982; Larick et al., 1987; Larick and Turner, 1990; Melton, 1990). The more unsaturated the fatty acid, the more susceptible it is to oxidation and a greater rate of autoxidation (Pearson et al., 1977) and consequently, the sensation of off flavors results. Effect of cookery on change in fatty acid composition has not been thoroughly
investigated. Most researchers have related raw fatty acid composition to cooked beef palatability attributes. However, CAST, 1991 reported cooking did not influence relative fatty acid composition of beef and Smith et al. (1989) found no consistent trend in fatty acid composition of beef steaks cooked to various degrees of doneness with different levels of subcutaneous fat.

It is widely recognized that as quality grade increases, lipid percentages increase (Savell et al., 1986). Therefore, steaks originating from higher quality grade carcasses, or anatomical regions within a carcass which possess a high degree of marbling, would likely have a higher caloric density (Rhee et al., 1982a; Berg et al., 1985; Renk et al., 1985; Hoelscher et al., 1988; Browning et al., 1990; Smith et al., 1989).

Because labelling certain nutrient properties such as cholesterol has been proposed by USDA and FDA and await implementation, it is imperative an accurate nutrient data base be established. Cholesterol values reported in the literature differ widely. Perhaps this stems from researchers using analytical procedures originally intended to quantify cholesterol in human serum, not muscle tissue. Therefore, our objectives were to develop a rapid, accurate and precise procedure using modern laboratory technology to determine CC in beef strip loin steaks both raw and cooked that differed in quality grade and also determine fatty acid and proximate composition and caloric content of these steaks.
MATERIALS AND METHODS

Selection of Strip Loins and Processing of Steaks. Cattle (steers) used in this study were genetically similar and fed a high concentrate corn-based finishing ration at the Rhodes Experiment Station, Rhodes, IA. Cattle were slaughtered at an average of 14.5 mo of age (USDA Standard-Average to High Choice) at a commercial packing plant, or at an average of 18.5 mo of age (USDA Prime) at the Iowa State University Meat Laboratory.

USDA yield and quality grades were determined 24 hr postmortem (PM) by three trained personnel from Iowa State University and one USDA Grader. Seven strip loins (I.M.P.S. 180) were removed 48 hr PM from each of the following USDA quality grade carcasses: Standard, low Select, high Select, low Choice and average to high Choice. These 35 strip loins were vacuum-packaged, stored 9d at 0°C to 2°C, cut into 3.0 cm thick steaks, frozen and stored at -20°C. The remaining seven Prime strip loins were stored for 23d at 0°C to 2°C, then cut into individual steaks, vacuum-packaged, frozen and stored at -20°C. Steaks were thawed 26 hr at 3°C prior to broiling, and subcutaneous fat was trimmed to a maximum thickness of 0.4 cm.

Anterior slices from each strip loin were carefully trimmed of all subcutaneous fat, perimysium and peripheral muscles so that only the completely trimmed longissimus muscle remained. Each slice was frozen with the use of liquid nitrogen and pulverized in a Waring Blender. The pulverized tissue was stored in Whirl-Pak bags and stored in a -20°C freezer until laboratory determinations could be made.

Lean from each strip loin steak was analyzed for percentage lipid extractable fat and percentage moisture following AOAC (1990) procedures. The
longissimus samples (about 3.5 gm) were loaded into oven-dried cotton thimbles, weighed and placed in a drying oven for at least 24 hr. Upon removal from the oven, all samples were reweighed (for determination of percentage moisture) and were subjected to lipid extraction using Soxhlet apparati and n-hexane as the solvent. After a 7 hr extraction period, samples were removed, air dried to remove most of the n-hexane, and placed in the drying oven for at least 24 hr before reweighing (for determination percentage of fat). All determinations were made in triplicate and only those that were within 5% of each other after computation to determine moisture and fat content were accepted (all others were reanalyzed until acceptable figures were obtained).

**Broiling Procedures.** Two broiling procedures were used in this study. The first method involved broiling steaks by using an electric consumer household oven broiler. Specifically, steaks were placed on broiler pans and cooked on the top rack where the meat surface was 10 cm from the heat source. Steaks were broiled at 246°C ± 14°C and first turned when the internal temperature reached 39°C. Internal temperatures were monitored with iron constantan thermocouples inserted into geometric center of each steak and attached to an Omega Digital Trendicator. Steaks were removed from the broiler at either 59°C or 65°C to account for post cooking temperature rise to achieve a final degree of doneness of either 62.8°C or 70°C.

The second method involved broiling steaks by using procedures typical to restaurant situations. Steaks were broiled in a large stainless steel electric broiler at 316°C ± 8°C. They were placed directly on the rack, turned at 39°C and removed when the internal temperature reached 57°C or 63°C to achieve a final degree of doneness of 62.8°C or 70°C respectively as monitored by use of thermocouples. After determining final internal temperatures, these steaks
were allowed to cool to room temperature (approx. 2 hr.) and prepared by methods described previously for the raw tissue slices before compositional analysis.

**Cholesterol Determination.** Frozen, liquid nitrogen pulverized tissue was accurately weighed directly into 15 x 150 mm test tubes which had clean teflon lined screw caps. Attention was given to each tube and cap to ensure neither was chipped, cracked or broken. These tubes were silanized prior to use (AOAC, 1976). An internal standard, 50 μl of deuterium (D) labelled cholesterol (Merck, Montreal, Canada), was added to each sample and standard tube. The procedure for sampling meat tissue is depicted schematically in Figure 1. Direct transesterification was initiated using modifications of procedures by LePage and Roy (1986) with the addition of 2 ml of methanol-benzene 4:1 (v/v) into each tube. Next 200 μl of acetyl chloride was added in 10 ml aliquots using a Hamilton syringe. This extremely reactive reagent enables refluxing to occur. A stream of nitrogen gas was used to replace air in the head space of each tube prior to closing screw caps down tightly and subjecting tubes to methanolysis at 100°C for 1 hr. Because acetyl chloride reacts violently with water, heating tubes in a water bath can cause explosions if cracks or chips are present in the tubes/caps. A safer alternative is to use an autoclave. After tubes cooled to room temperature, 2 ml of methanol and 5 ml of 6% potassium carbonate was added to each tube to stop the reaction and neutralize the mixture. Tubes were vortexed, capped and centrifuged at 3000 rpm for 10 minutes. An aliquot of the benzene upper phase was transferred to dram vials (approximately 3/4 full) by using a new disposable glass pipet for each tube. Dram vials were placed on a preheated block (85°C) and contents were dried completely under nitrogen gas. To form derivatives, 100 μl of acetonitrile (Regis Chemical, Morton Grove, IL)
and 100 μl of BSTFA (bis-trimethylsilylfluoroacetomide) (Regis Chemical) were added to each dried dram sample vial, capped with a teflon-lined screw top, vortexed and placed on the block heater for 1 hr at 65°C. Derivatized samples were transferred into injection vials (Sun Brokers, Wilmington, NC) using a new glass pipet for each vial. These were capped and placed in an auto injector model HP7673A (Hewlett-Packard, Avondale, PA) for analysis. A model HP5890 GC with a HP-1 column (crosslinked methyl silicone gum, 12.5 m x 0.2 mm x 11 μm film thickness) and a model HP5970A mass selective ion detector were used (Hewlett-Packard). A 1 μl volume was injected (splitless) into the injection port (300°C). Head pressure was 50 kPa and carrier gas (helium) flow rate was 1 ml/min. Initial oven temperature was 60°C with a ramp rate of 35°C/min to 300°C. Transfer line temperature was 300°C. Major ion fragments for cholesterol and 7-D labelled cholesterol were accomplished by using selective ion monitoring. Cholesterol was monitored at 368.3 atomic mass units (amu) and its 7-D isotope at 375.3 amu. A standard curve for cholesterol concentrations ranging from 25 mg/100 ml to 150 mg/ml provided a correlation coefficient of 0.995. These calculations are based on the ratio of known, purified cholesterol (Sigma-St. Louis, MO) relative to the internal standard (7-D cholesterol) as determined by peak heights from chromatograph data. Thus the ratio of an unknown sample to internal standard was back calculated relative to the ratio of known, purified cholesterol and internal standard. Data was then converted to (mg/100 gm) tissue equivalents. All determinations were made in duplicate and only those values within 5% of each other after computation were accepted (all others were re-analyzed until acceptable values were reached).
To assess cholesterol recovery, (extraction efficiency) a known amount of purified cholesterol was added to a meat sample. Therefore, CC of the meat sample and CC of the meat sample plus added purified cholesterol was determined to assess recovery using these methods. In three samples done in triplicate, we found 97.6 to 102.2 percent recovery for our spiked samples.

**Fatty Acid Determination.** Fatty acids were extracted simultaneously with cholesterol. An aliquot of the benzene upper phase was transferred to dram vials (approximately 3/4 full) by using a new disposable glass pipet for each tube. A Water’s model Dimension 1 gas chromatograph (Milford, MA) equipped with a flame ionization detector was used with a 30 m capillary column (0.25 mm internal diameter, DB-225 stationary phase with a film thickness of 0.25 µm, J&W Scientific, Folsom, CA). Operating conditions were: helium carrier gas flow of 70 ml/min., inlet temperature of 210°C head pressure of 50kPa, and detector temperature of 250°C. A dynatech precision GC 411V autosampler (Baton Rouge, LA) was used for injecting 1.5 µl sample into a split injection port (1:20). Fatty acid methyl esters were identified by comparison with methyl ester standards (NuChek Prep, Elysian, MN). Duplicate samples of FAME peaks were quantified by electronic integration based on peak area.

**Protein Content.** Protein content was determined by the difference method. Since we determined lipid and moisture content, we used ash values reported in Nutrient Values of Muscle Foods (National Live Stock and Meat Board, 1990) from raw and cooked strip loin steak data. These values were 1.0% and 1.3% for raw and cooked, respectively. Therefore raw protein percentage = 99 - (% Lipid) - (% Moisture); cooked protein percentage = 98.7 - (% Lipid) - (% Moisture).
Caloric Content. Caloric content (based on the protein and lipid content of raw and cooked muscles was calculated by the Atwater conversion method (Bogert et al., 1983) using the following equation: Total Kcal = (gm Protein x 4) + (gm Lipid x 9).

Statistical Analyses. General linear models analysis of variance procedures and correlation and regression techniques (SAS, 1990) were used on the data. Variables included quality grade, internal temperature and broiler temperature as main effects and all possible combinations of these three variables for two-way and the three-way interactions. None of the interactions were significant. Fischer's least significant difference procedure was used for mean separation.
Figure 1. Schematic diagram of the procedure for sampling muscle (meat) tissue to determine cholesterol content and fatty acid composition.
RESULTS AND DISCUSSION

Data on CC of beef strip loin steaks stratified by quality grade are presented in Table 1. The mean CC ranged from 48.3 to 56.2 mg/100 gm lean and are expressed on an "as-is" or wet weight basis similar to how steaks would be consumed. Lean from steaks of Standard and low Select had a lower (p<.05) CC than Prime; no significant differences were found for steaks from Choice and high Select or Choice and Prime. These data are in close agreement with Rhee et al. (1982a) who reported longissimus muscle (LD) steaks possessing a practically devoid marbling degree had a lower CC than those containing a higher degree of marbling. In addition, Hoelscher et al. (1988) evaluated effects of marbling degree and found as steaks increased in quality grade from Select to Prime, a numerical, but not significant increase in CC was observed. Though our trends are similar to results reported by other investigators, our absolute values are about 12 to 15 percent lower. One explanation is that many researchers have used the colorimetric procedure of Searcy and Berquist (1960) with slight modifications. This procedure was originally intended to quantify CC in serum. Other interfering compounds may have reacted colorimetrically which would tend to overestimate the true CC.

The procedure of Searcy and Berquist, (1960) was an improvement over the Liebermann Burchard reaction (Schoenheimer and Sperry, 1934; Sperry and Webb, 1950) and Zlatkis procedure (Zlatkis et al., 1953). These procedures also overestimated CC because of interfering compounds such as sterols (lanosterol, campesterol, sitosterol, stigmasterol, progesterone, testosterone, estradiol, dehydroisoandrostenone, fucosterol, desmosterol, coprosterol), and
unsaturated fatty acids reacted colorimetrically. In a comparison of colorimetric, enzymatic and gas-liquid chromatography (GLC) procedures, Lillienberg and Svanborg (1976), reported the GLC procedure gave values 12% lower than those for colorimetric determinations, while enzymatic methods were closer in agreement to GLC. However, Nakagawa et al. (1979) found the presence of neutral sterols in foods containing cholesterol interfered with the determination of true CC when using enzymatic procedures. In addition, Ulberth and Reich (1992) compared enzymatic and GLC methods for determining CC. They found that the enzymatic determination of sterols for cholesterol is not specific for cholesterol. Sterols, with a 3-β-OH group react with the enzyme. They (Ulberth and Reich, 1992) concluded that the enzymatic method when applied to foods containing animal fats, overestimates the amount true cholesterol present. Because of potential interferences from extraneous compounds which may mimic cholesterol by colorimetric or enzymatic techniques, chromatographic methods are the preferred method to enhance accuracy and precision in foods (Punwar, 1975; Lillienberg and Svanborg, 1976; Sheppard et al., 1977; Nakagawa et al., 1979; Kaneda et al. 1980, Kuo et al., 1992; Ulberth and Reich, 1992). Our data numerically closely corresponded with that reported by Hood and Allen (1971). They reported CC ranging from 46 to 57 mg/100 for samples which had 4.0 or 7.3 percent lipid in the LD, respectively. In addition, Khatri (1992) reported CC in LD from steers ranged from 49-56 mg/100 gm with corresponding lipid contents of 2 to 8.0 percent.

The regression equation for mg cholesterol/100 gm LD (Y) on % fat (X) was $Y = 48.2 + 0.69X$. This equation is in close agreement to that of Khatri (1992)
for LD and Tu et al. (1967) who used this equation for a composite consisting of LD, semimembranosus, serratus ventralis, semitendinosus and psoas major muscles. They found $Y = 48.9 + 0.72X$ and $Y = 48.9 + 1.7X$, respectively. Rhee et al. (1982a) reported an equation for LD which had a slightly higher intercept ($Y + 54.73 + 0.87X$). We found a correlation coefficient ($R^2$) between lipid content and CC of 0.40. This was nearly identical to Rhee et al. (1982a) and Khatri (1992).

The values for CC of cooked steaks as influenced by quality grade, broiling temperature and degree of doneness are reported in Table 1. Though there were no significant interactions, the data is presented in this manner because this was a unique approach which encompassed quality grade and cookery considerations. No differences for the main effect of quality grade were found in CC of LD from cooked steaks ranging from Standard to Prime. However, broiled steaks had higher ($p<.01$) CC in cook lean than in raw. This would be expected from evaporative losses during cooking which concentrates lipid compounds and decreases moisture content. These data agree with Rhee et al. (1982a) who reported no differences among quality grades when steaks were cooked to $60^\circ C$ or $75^\circ C$. However, Hoelscher et al. (1988) observed after cooking, Prime steaks had a higher CC than Choice or Select. Berg et al. (1985) showed that on a dry-weight basis, steaks which contained a practically devoid marbling degree had a higher CC than those with a modest or moderate degree of marbling. It is more difficult to compare their data to most previously published studies because it was reported on a dry matter basis. Because steaks are consumed on a wet-weight basis, it seems logical that data bases be developed on typical food consumption practices.
The effect of final internal temperature on CC across all quality grades and both broiling temperatures is presented in Figure 2. Steaks heated to 62.8°C had a lower (p<.05) CC than those heated to a 70°C final internal temperature. This agrees with Rhee et al. (1982a) who reported higher CC in steaks heated to 75°C compared with 60°C and Kregel et al. (1986) who found ground beef heated to 77°C had a higher CC than patties heated to 71°C. This is related to evaporative losses and resulting moisture percentage and lipid compounds in the cooked steaks.

The effect of broiling temperature on CC of lean from steaks across all quality grades and both degrees of doneness is presented in Figure 3. No differences (p>.05) in CC were observed, regardless of whether steaks were broiled at 246°C or 316°C. These temperatures were chosen because they are similar to those used by consumers in a household oven broiler or by commercial restaurants, respectively. Little published data is available to compare these data. Berg et al. (1985) compared effects of broiling and microwaving on beef rib steaks, and they reported broiled rib steaks had a significantly lower CC than those cooked by microwaving. This was likely due to greater evaporative losses and concentration of lipid compounds for microwaved steaks.

Effect of beef quality grade on fatty acid composition of lean from raw strip loin steaks is reported in Table 2. Steaks from Standard grade strip loins had a lower (p<.05) percentage of C 16:0 and higher (p<.05) percentage of C 18:2 than steaks from other quality grades. Differences were not observed (p>.05) among quality grades for other fatty acids analyzed. These results compare favorably with Eichhorn et al. (1985) who evaluated steaks from bulls and steers. They postulated that increases in intramuscular fat deposition, as was
observed in steers, decreased the relative percentage of phospholipids which were primarily present as polyunsaturated fatty acids. This concept seems to be supported by Mills et al. (1992) who reported lean from steers slaughtered at time of initiating the feeding trial (presumably when marbling was low) had higher relative percentages of C18:2 compared with samples from steers taken at the conclusion of the trial. Furthermore, Smith et al. (1989) observed that lean from tenderloin roasts and clod and top round steaks was numerically higher in all cases for C18:2 from Standard grade carcasses compared with Select and Choice. Though not statistically different, this was most likely because of having only three observations per quality grade in that study (Smith et al., 1989).

Effect of quality grade on fatty acid composition of lean from broiled strip loin steaks is reported in Table 3. Steaks from Standard and low Select strip loins had a higher (p<.05) percentage of C18:0 and C18:2 lower (p<.05) percentage of C18:1 than the steaks from Choice and Prime. No other differences were due to effect of quality grade (p>.05) for other fatty acids analyzed. Interestingly, the observed range for C18:2 expressed as a relative percentage in raw lean was 9.3 (Standard) to 3.5 (Prime) compared with 3.6 (Standard) to 2.5 (Prime) for cooked. The greater the degree of unsaturation of fatty acids, the lower the melting point of the lipid. The lower melting point of unsaturated fatty acids compared with that for saturated fatty acids results in greater losses of lipid during cooking (CAST, 1991). This may partially explain these observed differences for C18:2 in our study. Values reported for cooked lean, in general, are similar to those reported for raw. This agrees with results published by Smith et al. (1989) and CAST (1991) who concluded that fatty acid profile seems to be changed little by cooking.
Effect of degree of doneness on fatty acid composition of lean from beef strip loin steak is reported in Table 4. We found steaks broiled to 70°C had a lower (p<.05) percentage of C 18:0 than those cooked to 62.8°C. No other differences were significant (p>.05). Little information exists in the published literature to compare these data. However, our findings were in close agreement with those of Smith et al. (1989) who cooked beef cuts to rare, medium and well done internal endpoints.

Effect of broiling temperature on fatty acid composition of lean from strip loin steaks reported in Table 5. Steaks broiled at 246°C had higher (p<.05) relative percentages of C 14:0, C 16:0 and C 18:2 and less (p<.05) C 18:1 than those broiled and 316°C. Again, little published information is available to compare our results to those of others for effect of cooking temperature.

Effect of quality grade on proximate composition of raw and cooked lean from beef strip loin steaks is reported in Table 6. In raw and cooked steaks as quality increases, LC increases (p<.05) and MC decreases (p<.05). Protein is not affected (p<.05). Significant differences (p<.01) for LC, PC, and MC were observed from raw to cooked among all quality grades. This data is in agreement with those reported by Rhee et al. (1982a), Berg et al. (1985), Savell et al. (1986), Hoelscher et al. (1988), and Smith et al. (1989).

Effect of degree of doneness on proximate composition of lean from strip loin steaks is reported in Table 7. Steaks broiled to a medium-rare final internal temperature (62.8°C) had a lower (p<.05) LC and PC and higher (p<.05) MC than those heated to 70°C. Our findings are similar to those reported by Rhee et al. (1982) and Smith et al. (1989) who found that heating to a higher final temperature increased evaporative losses (primarily MC) and concentrated the lipid and protein in the cooked steaks.
Effect of broiler temperature on proximate composition of lean from strip loin steaks is presented in Table 8. We found that lean from steaks broiled at 246°C had a lower (p<.05) LC and higher (p<.05) MC than those heated at 316°C. Little or no previously published data exists to compare effects of broiling beef in a household oven compared with procedures used commonly by restaurants.

Effects of quality grade, degree of doneness and broiler temperature on calculated caloric content are presented in Figures 4, 5 and 6, respectively. Our findings indicated that as quality grade and degree of doneness increased, caloric density was higher (p<.05). Broiler temperature had less (p>.05) influence on caloric density. These observations are similar to those reported by Berg et al. (1985) and Browning et al. (1990).
Results of this study suggest that more rapid and accurate procedures to extract and quantify CC from tissue must be given consideration. Because most research laboratories are equipped with or have access to GCMS equipment, these procedures could easily be adopted. Furthermore, differences observed in CC of raw steaks among quality grades were negated after cooking. Therefore, selecting steaks for leanness in hopes of reducing cholesterol intake will not fulfill consumer expectations.

Subjective quality grade classifications can be an effective means in sorting differences related to certain nutritional concerns, namely fat and calories. Even though our results indicated few significant differences for fatty acid profiles were related to quality grade and cookery, the magnitude of the differences was perhaps too small to be practical. Furthermore, as quality grade increases values for LC and calculated caloric density increase. Consumers desiring to reduce fat and calorie have alternatives and can choose beef that has less marbling.
Table 1. Effects of USDA quality grade and broiling on cholesterol content of raw and cooked lean from beef strip loin steaks

<table>
<thead>
<tr>
<th>Quality Grade</th>
<th>Raw</th>
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<th>70°C</th>
<th>62.8°C</th>
<th>70°C</th>
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<tr>
<td>Standard (n=7)</td>
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<td>60.3</td>
<td>63.8</td>
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<td>60.4</td>
<td>63.4</td>
</tr>
<tr>
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<td>64.2</td>
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<tr>
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<td>64.3</td>
<td>64.0</td>
<td>63.8</td>
<td>69.0</td>
</tr>
<tr>
<td>Ave-Hi Choice (n=7)</td>
<td>53.8a,b</td>
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<td>64.0</td>
<td>63.5</td>
<td>66.6</td>
</tr>
<tr>
<td>Prime (n=7)</td>
<td>56.2b</td>
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<td>69.2</td>
<td>62.8</td>
<td>67.1</td>
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<tr>
<td>SE</td>
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Means within a column with different superscripts differ (p<0.05).

Table 2. Effect of USDA beef quality grade on fatty acid composition of raw beef strip loin steaks

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<tr>
<th>Quality Grade</th>
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<th>18:1</th>
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<tr>
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<td>Tr</td>
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<td>3.6</td>
<td>Tr</td>
<td>14.3</td>
<td>43.1</td>
<td>5.9b</td>
<td>Tr</td>
</tr>
<tr>
<td>High Select (n=7)</td>
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<td>Tr</td>
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<td>Tr</td>
<td>14.2</td>
<td>42.8</td>
<td>4.8b</td>
<td>Tr</td>
</tr>
<tr>
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<td>Tr</td>
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<td>Tr</td>
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</table>

Means within a column with different superscripts differ (p<.05).
Table 3. Effect of USDA beef quality grade on fatty acid composition of broiled beef strip loin steaks

<table>
<thead>
<tr>
<th>Quality Grade</th>
<th>Fatty Acid%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
</tr>
<tr>
<td>Standard (n=7)</td>
<td>3.1</td>
</tr>
<tr>
<td>Low Select (n=7)</td>
<td>3.5</td>
</tr>
<tr>
<td>High Select (n=7)</td>
<td>3.3</td>
</tr>
<tr>
<td>Low Choice (n=7)</td>
<td>3.4</td>
</tr>
<tr>
<td>Ave-Hi Choice (n=7)</td>
<td>3.2</td>
</tr>
<tr>
<td>Prime (n=7)</td>
<td>3.5</td>
</tr>
<tr>
<td>SE</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (p<.05).

Table 4. Effect of degree of doneness on fatty acid composition of beef strip loin steaks from all quality grades broiled at different temperatures

<table>
<thead>
<tr>
<th>Degree of Doneness</th>
<th>Fatty Acid%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
</tr>
<tr>
<td>62.8°C (n=84)</td>
<td>3.2</td>
</tr>
<tr>
<td>70°C (n=84)</td>
<td>3.4</td>
</tr>
<tr>
<td>SE</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (p<.05).

Table 5. Effect of broiler temperature on fatty acid composition of beef strip loins from all quality grades cooked to different temperatures

<table>
<thead>
<tr>
<th>Broiler Temperature</th>
<th>Fatty Acid%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
</tr>
<tr>
<td>246°C (n=84)</td>
<td>3.5</td>
</tr>
<tr>
<td>316°C (n=84)</td>
<td>3.2</td>
</tr>
<tr>
<td>SE</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts differ (p<.05).
### Table 6. Effect of USDA beef quality grade on proximate composition of raw and cooked beef strip loin steaks

<table>
<thead>
<tr>
<th>Marbling Degree</th>
<th>%Lipid Raw</th>
<th>%Protein Raw</th>
<th>%Moisture Raw</th>
<th>%Lipid Cooked</th>
<th>%Protein Cooked</th>
<th>%Moisture Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>2.2a</td>
<td>23.5</td>
<td>73.8a</td>
<td>4.3a</td>
<td>29.1</td>
<td>65.3a</td>
</tr>
<tr>
<td>Low Select</td>
<td>3.4b</td>
<td>23.0</td>
<td>72.6b</td>
<td>5.8b</td>
<td>27.8</td>
<td>65.1a</td>
</tr>
<tr>
<td>High Select</td>
<td>4.1b,c</td>
<td>23.2</td>
<td>72.2b</td>
<td>7.4c</td>
<td>28.0</td>
<td>63.4b</td>
</tr>
<tr>
<td>Low Choice</td>
<td>5.4c</td>
<td>23.2</td>
<td>70.9c</td>
<td>8.2c,d</td>
<td>28.2</td>
<td>62.4b</td>
</tr>
<tr>
<td>Ave-High Choice</td>
<td>7.2d</td>
<td>23.0</td>
<td>69.3d</td>
<td>11.4e</td>
<td>27.0</td>
<td>60.4c</td>
</tr>
<tr>
<td>Low-Ave Prime</td>
<td>10.5e</td>
<td>23.2</td>
<td>65.8e</td>
<td>13.6f</td>
<td>27.1</td>
<td>58.1d</td>
</tr>
<tr>
<td>SE</td>
<td>0.6</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

* a,b,c,d,e Means within a column with different superscripts differ (p<0.05).

* x Seven observations per mean reported.

* y Twenty-eight observations per mean reported.

### Table 7. Effect of degree of doneness on proximate composition of beef strip loin steaks from all quality grades broiled at different temperatures

<table>
<thead>
<tr>
<th>Degree of Doneness</th>
<th>%Lipid</th>
<th>%Protein</th>
<th>%Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.8°C (n=84)</td>
<td>8.1a</td>
<td>27.2a</td>
<td>63.4a</td>
</tr>
<tr>
<td>70.0°C (n=84)</td>
<td>9.0b</td>
<td>28.3b</td>
<td>61.5b</td>
</tr>
<tr>
<td>SE</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* a,b Means within a column with different superscripts differ (p<.05).

### Table 8. Effects of broiler temperature on proximate composition of beef strip loin steaks from all quality grades cooked to different internal temperatures

<table>
<thead>
<tr>
<th>Broiler Temperature</th>
<th>%Lipid</th>
<th>%Protein</th>
<th>%Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>246°C (n=84)</td>
<td>8.1a</td>
<td>27.5</td>
<td>62.9a</td>
</tr>
<tr>
<td>316°C (n=84)</td>
<td>8.9b</td>
<td>27.6</td>
<td>62.0b</td>
</tr>
<tr>
<td>SE</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* a,b Means within a column with different superscripts differ (p<.05).
Figure 2. Effect of final internal temperature on cholesterol content of beef strip loin steaks from all quality grades.

a,b Means with different superscripts differ (p<.05).
Figure 3. Effect of broiling temperature on cholesterol content of beef strip loin steaks from all quality grades.
Figure 4. Effect of quality grade on calculated caloric content of broiled beef strip loin steaks

a,b,c,d,e Means with different superscripts differ (p<.05).

x Each bar is the mean of 28 observations.
Figure 5. Effect of degree of doneness on calculated caloric content of broiled beef strip loin steaks

a,b Means with different superscripts differ (p<0.05).
Figure 6. Effect of broiler temperature on calculated caloric content of beef strip loin steaks
REFERENCES CITED


GENERAL SUMMARY

Strip loin steaks of various USDA quality grades, Prime, Choice, Select and Standard, were selected from cattle of known chronological age, feeding regimen, management and common ancestry. The objective of this part of the study was to determine the effects of intramuscular fat (marbling) on palatability and the effects of two broiler temperatures and two degrees of doneness across degrees of marbling on palatability of beef loin steaks. In this study steaks from Prime grade carcasses were found to be the most palatable and those from Standard grade carcasses were the least palatable. Loin steaks from Choice and Select grade carcasses were similar in their palatability attributes. Based on these palatability results, it seems premiums should be rewarded for Prime grade and discounts accorded to Standard grade carcasses relative to Choice prices. On the other hand, discounting Select relative to Choice seems unwarranted.

Steaks of various quality grades broiled to an internal temperature of 62.8°C (medium-rare) scored higher for all palatability attributes than those broiled to 70°C regardless of quality grade. Of the palatability attributes studied, only juiciness scores were higher for steaks broiled at a broiler temperature of 246°C than those broiled at 316°C. Consequently, internal degree of doneness of loin steaks is a more important effector of palatability than broiler temperature. Furthermore, a lower internal degree of doneness of loin steaks allows steaks with low degrees of marbling, such as Select, to have acceptably palatable eating characteristics.

A new method of determining cholesterol of loin steaks varying in quality grade was used in this study, gas chromatography-mass spectrometry
(GCMS). Unlike commonly used colorimetric methods which seemingly overestimate cholesterol due to interfering substances, the GCMS procedure is specific for cholesterol. Consequently, the results of this study for cholesterol content of raw loin steaks of various quality grades were 10-15 percent lower than most other reported studies. Although cholesterol content was higher in higher quality grades, no significant differences were observed on cholesterol content due to quality grade except in the Prime grade. Steaks broiled to 62.8°C doneness however, had less cholesterol than those broiled to 70°C. The lower amount of cholesterol in steaks at 62.8°C relative to 700°C was interpreted as being due to less moisture loss during cookery. Also cooked cholesterol values were about 20 percent higher than raw values due principally to moisture loss during cookery. Indeed, cookery negated differences in cholesterol content observed in raw beef steaks among various quality grades.

Though significant differences for a few fatty acids were determined, the magnitude of these differences was quite small, and not related to quality grade. However, lipid content and caloric density were related to quality grade. Lipid content became more concentrated after cooking and was most apparent at the higher degree of doneness. Consequently, selection of leaner beef cuts with less marbling will reduce both intake of fat and calories. Moreover, external fat trim had no significant relationship to lipid and cholesterol content or caloric density. Therefore, trimming subcutaneous fat prior to broiling will have little influence on cholesterol, total fat or calories ingested from consuming the cut.

In conclusion, palatability of beef steaks can be optimized by broiling to a medium-rare degree of doneness, regardless of quality grade. Also, the use of GCMS to determine cholesterol provides a more accurate assessment of
cholesterol content of beef loin steaks. That is, cholesterol content was found to be 10-15 percent lower than reported in the current nutrient handbooks, because of the methods specificity for determining cholesterol content of beef loin steaks.
GENERAL LITERATURE CITED


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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my parents, Dean and Priscilla Johnson, for their love and belief in me during this program. In addition, I would also like to thank Dr. F.C. Parrish, Jr. and his wife Fern who provided considerable advice, guidance and friendship during my program. I appreciate the many opportunities you have provided me to grow professionally by allowing my participation in numerous teaching, coaching and research opportunities. Furthermore for initiating greater participation of graduate students in AMSA committees. I thank you for involving me in IMPA functions as well as numerous related meat extension events which enabled me considerable experiences not commonly afforded to many students. I also thank you for nominating me for several awards and honorary societies. Furthermore I appreciate the suggestions and encouragement from my committee members: Dr. Dennis Olson; Dr. Steve Nissen; Dr. Ken Prusa; and Dr. Paul Hinz. I would also like to thank Dr. Gene Rouse for providing certain research opportunities.

Special thanks are extended to Mr. and Mrs. Eric Smith for their friendship as well as Dan Schaefer, Dennis Gruber, Elisabeth Huff, Rich Hall, Dirk Beekman, Mark Kreul, Bryan Reiling, Steve Lonergan, Adonna Knight and other office mates, including Kyle Holland.

Thanks must go to Jim Duff, Rich Hall, Teresa Stumpf, Janis Brownlee and Craig Morris for assisting me in aspects related to meat preparation. In addition, I appreciate advice from Greg Link, John Rathmacher, Deb Webb and Marcia King-Brink for analytical procedures. I would also like to thank the
Meat Lab staff: Randy Petersohn, Jerry Knight, Jim O'Brien, Mike Holtzbauer and Dick Foster and others for helping facilitate research, teaching and meat judging. I am grateful to Lisa Mayberry and Sharon Coletti for their assistance and advice. Also, I thank Bob Rust for allowing me to participate in shortcourses dealing with meat industry issues.

I would also like to thank Phil Core of Monfort of Des Moines and Steve Pearson from Beef Specialist's of Iowa for providing me with teaching and research opportunities. Without their cooperation, collection of research data and conducting beef workouts for the judging team would have been difficult.

I would also like to thank former students and meat judging team members for allowing me the privilege to have some influence on their lives and they my own. I was very humbled to have the Block and Bridle Club dedicate the Little North American to me and be recipient of a Teaching Excellence Award from Iowa State University.

A sincere thanks must go to Pam Smith for her professionalism and patience. She made several evening and weekend sacrifices to accommodate my schedule in the preparation of this dissertation.

I am deeply indebted to my wife Renee. She has endured a great deal. With my frequent 4:30 am departures to packing plants, overnight stays, long hours, deadlines and employment search, she was always there for me, even though she had two jobs, a full load of classes and wedding plans to coordinate. From the bottom of my heart, thanks Renee.
APPENDIX I. SENSORY PANEL EVALUATION FORM
## Sensory Evaluation of Beef Steak

<table>
<thead>
<tr>
<th>Tenderness Score</th>
<th>Juiciness Score</th>
<th>Flavor Intensity Score</th>
<th>Flavor Score</th>
<th>Overall Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Extremely tender</td>
<td>8 Extremely juicy</td>
<td>8 Extremely intense</td>
<td>8 Extremely flavorful</td>
<td>8 Extremely palatable</td>
</tr>
<tr>
<td>7 Very tender</td>
<td>7 Very juicy</td>
<td>7 Very intense</td>
<td>7 Very flavorful</td>
<td>7 Very palatable</td>
</tr>
<tr>
<td>6 Moderately tender</td>
<td>6 Moderately juicy</td>
<td>6 Moderately intense</td>
<td>6 Moderately flavorful</td>
<td>6 Moderately palatable</td>
</tr>
<tr>
<td>5 Slightly tender</td>
<td>5 Slightly juicy</td>
<td>5 Slightly intense</td>
<td>5 Slightly flavorful</td>
<td>5 Slightly palatable</td>
</tr>
<tr>
<td>4 Slightly tough</td>
<td>4 Slightly dry</td>
<td>4 Slightly bland</td>
<td>4 Slightly off-flavor</td>
<td>4 Slightly unpalatable</td>
</tr>
<tr>
<td>3 Moderately tough</td>
<td>3 Moderately dry</td>
<td>3 Moderately bland</td>
<td>3 Moderately off-flavor</td>
<td>3 Moderately unpalatable</td>
</tr>
<tr>
<td>2 Very tough</td>
<td>2 Very dry</td>
<td>2 Very bland</td>
<td>2 Very off-flavor</td>
<td>2 Very unpalatable</td>
</tr>
<tr>
<td>1 Extremely tough</td>
<td>1 Extremely dry</td>
<td>1 Extremely bland</td>
<td>1 Extremely off-flavor</td>
<td>1 Extremely unpalatable</td>
</tr>
</tbody>
</table>

### Comments:
APPENDIX II. FAT/MOISTURE DETERMINATION PROCEDURES
Moisture/Fat Determination

Theory:
The sample is weighed, dried, cooled, and then reweighed. The weight loss is calculated as moisture content. The sample is then used for fat determination where the dried sample is placed on the fat extractor and fat is removed using petroleum ether. The sample is extracted, dried and reweighed to determine fat content.

Equipment and Chemicals:
- Whatman 22 x 80 cellulose extraction thimbles (dried at least 2 hours and stored in desiccator)
- Weighing spatulas
- Balance
- Drying oven
- Soxhlet fat extractor
- Petroleum ether
- Cotton
- Desiccator

Procedure:
1) Weigh dried thimble, record weight (A), and tare the balance. The thimbles collect moisture, so work rapidly and do not touch them with your hands. If the samples are >20% fat, dried cotton should be put in the bottom of the thimble and included in the thimble weight. This is done to absorb any fat that could leak out during the initial drying of the sample.

2) Using a spatula, transfer 5g of ground, emulsified, or blended sample into the tared thimble and record the weight (B).

3) Place sample in drying oven for at least 18 hours.

4) Remove samples from oven and allow to cool in a desiccator and weigh to get the dried weight (C).

5) Run the dried samples on the Soxhlet fat extractor for at least 6 hours.

6) After extraction, samples should be dried in the drying oven for at least 2 hours.

7) Remove samples from oven and allow to cool in a desiccator and weigh to get the extracted weight (D). The samples can then be discarded.
Calculations:

\[
\text{% Moisture} = \frac{B - (C - A)}{B} \times 100
\]

\[
\text{% Fat} = \frac{C - A - (D - A)}{B} \times 100
\]
APPENDIX III. CHOLESTEROL AND FATTY ACID PROCEDURES
STANDARD OPERATING PROCEDURE

TITLE: Cholesterol, Free fatty acid transesterification method
Computer File Location: c:sop\cholanal

1.0 PURPOSE:
The purpose of this SOP is to provide instruction and outline a method of analyzing for cholesterol and free fatty acids in tissue samples.

2.0 APPLICATION:
This method can be applied when it is desired to determine cholesterol levels of samples. It is, however, a very involved assay that is easily ruined by contamination problems and one that uses hazardous chemicals extensively.

3.0 REFERENCES:

4.0 ASSOCIATED SOPs:
4.1 Procedures:
4.2 Reagents:
4.3 Others:

5.0 SUPPLIES:
5.1 ITEM                     LAB. LOCATION                     SUPPLIER
1.Disposable glass pipette  drawer, Rm. 329                     Chem Stores
2.Eppendorf gun and tips    drawer, Rm. 329                     Fisher
3.Autoclave                 Rm. 329 or 330                     Fisher
4.Test tubes: 16x125mm (not 13x100mm)oven, Rm. 330
5.Mass spec vials and caps  drawer, Rm. 329                     Chem Stores
6.Heating block w/gas needles Rm. 330
7.2 Methanol wash buckets   Rm. 330
8.Thin spatula for weighing  drawer, Rm. 329                     Fisher
9.Aspirator                 drawer, Rm. 330                     Fisher
10.Centrifuge               Rm. 330
11.Vortex mixer             Rm. 329 or 330                     Fisher
12.Reagent bottles with reagent bottle tops
13.Acetonitrile (ACN)       upper shelves, Rm. 329             Regis
14.BSTFA, derivatizing agent *                                    Regis
15.Hamilton syringe (one each for ACN and BSTFA)

SOP# 118, c:sop\cholanal
ITEM |
<table>
<thead>
<tr>
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</thead>
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<tr>
<td>16. Kim wipes</td>
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<tr>
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LAB. LOCATION |
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SUPPLIER |
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</tr>
<tr>
<td>chemistry stores</td>
</tr>
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<td>chemistry stores</td>
</tr>
</tbody>
</table>

6.0 PROCEDURE:

6.1 Step by step procedure:

Possible Sources of Contamination:

Touching telephone, drawer handle, keyboard, water fountain, nitrogen tank, or anything else in general use in lab. Contamination will invariably jump into samples at every opportunity. A quick rinse of Methanol should clean gloves if ever in doubt. Do not immerse, however, because Methanol will get through to your hands.

Try to avoid breathing or talking over samples, or leaving them uncapped and unattended.

STEP 1: WEIGHING UP SAMPLES

You will need 15x150mm test tubes with screw caps, one for each sample and standard. Select tubes which can be easily labeled, and which are not chipped, cracked, or broken. Also select caps which are not cracked or broken, and which have a clean teflon lining. The newer the cap the better. Careful selection of tubes and caps will pay off later in assay, when vortexing and heating.

Rinse test tubes, caps, 1"x1" aluminum foil, thin spatula, scissors, and test tube rack in methanol. Using Kim wipes dipped in Methanol, clean weighing scale and table. Wipe all surfaces which may be touched by equipment or fingers, such as the zeroing mechanism on scale, scale door, and especially the scale itself.

Weigh samples directly into the tube. Keep the weights within a range of 0.0375 to 0.0475. Accuracy of weights is critical to final analysis results; some ideas about weighing can be helpful.
1. Weigh up to target weight range. If you go over target range, start over rather than attempt to remove sample.

2. Treat all samples consistently. Cap tubes after each weight.

STEP 2: PREPARING STANDARD TUBES

The internal standard is kept frozen and the cholesterol and free fatty acid standards are kept cool in the cold room. They need to be thawed and brought to room temperature in a consistent manner as volumes will fluctuate as a function of temperature. One hour thaw time has been used. Each flask contains a magnetic stir bar and each sample is stirred on a stir plate for about five minutes.

Because the standards are relatively expensive, it is important to take great care in ensuring that they not be contaminated. When uncapping test tubes, keep the caps in order so that the same caps are used on each tube. Standards are handled in the following manner to best protect them from sources of contamination:

1. The proper amount of FFA std. is first poured into clean 10ml beaker.
2. The 1.25ml Eppendorf tip is used and eppendorf gun set on position 4 (1 = 25ul so 4 = 100ul).
3. One shot of FFA std in each FFA CHOL std tube.
4. Discard remainder of FFA std which is in eppendorf tip and/or beaker.
5. Repeat steps 1-2 using Cholesterol std, new clean beaker and eppendorf tip.
6. Set Eppendorf Gun to position 3 (3 = 75ul).
7. One shot in each FFA CHOL std tube and one shot each in CHOL INT std tubes.
8. Cap FFA CHOL std tubes.
9. Repeat steps 1-2 using Internal std, new clean beaker and eppendorf tip.
10. Set gun to 2 (2 = 50ul).
11. One shot each in CHOL INT tubes and INT std tubes.
12. Cap remaining standard tubes.
14. Uncap meat sample tubes (not blanks or standards).
15. One shot Int std into each meat sample tube, recap.
16. Refreeze and chill standards.

It is important not to waste standards and to be sure of amount needed before using them. Best to practice with water and other eppendorf tips before recklessly pouring standards into beakers. Even though beakers are cleaned and rinsed with Methanol, it is not worth the possibility of contaminating standards by pouring unused back into flasks. It is for the same reason that we do not insert eppendorf tips directly into them.
STEP 3: METHANOL-BENZENE (2ml)

Before adding MeOH-Benzene, wipe spout well with Methanol in case it has been touched or rested against something which could contaminate it. Be sure reagent is set at 2 ml. Remove bubbles by squirting some in sink. Keep caps in order after removing them from test tubes. First add 2 ml to meat sample tubes, being careful not to touch any tubes with the spigot. Recap tubes. Do the same with the blanks, then the INT stds, INT CHOL stds, and finally FFA CHOL stds.

STEP 4: ACETYL CHLORIDE (300ul)

Acetyl Chloride is a reagent which enables refluxing to occur. It is extremely reactive with water. It is not known what would happen should a drop of it get on your skin or in your eyes, but you can probably imagine something grotesque. BE CAREFUL! Use a fume hood.

Although there should be no water in your samples, the tubes sometimes do pop. Use a large test tube rack and do a few tubes at a time. Again, keep the caps in order. Point the tubes away from yourself and away from each other so that they do not cross-contaminate if something should pop out. Add 10ul at a time to each tube using the small Hamilton syringes and small beaker. A full Hamilton syringe (500ul) will actually hold only about 440-450ul. If you add to four test tubes at a time, it is easy to keep them pointed away from you and from each other, each "round" of 10 shots (10ul each) will add 100ul to each tube. Use three "full" eppendorf tips for each 4 test tubes to add the full amount (300 ul). Use two hands to steadily add acetyl chloride. Again, be careful not to touch test tubes with the tip, and add it so that it gently runs down the side of the tube.

VORTEX AND MAKE SURE THE CAPS ARE TIGHT

STEP 5 AND 6: REFLUXING AND COOLING SAMPLES

Since Acetyl chloride reacts violently with water, heating the tubes in the water bath could cause explosions. An alternative is the autoclave, but it must be regularly monitored. The temperature is difficult to set at 100°C (red scale), but it can be used +/-5°C. The pressure reducing/increasing valve underneath is used to attain about 103°C, when in sterilization mode. After one hour, liquid cool for 10-15 min. and then vent for 5 min. Further cool samples in room temperature water bath after removing from autoclave.

We will be using the autoclave that is down in the metabolism barn. It should be set on liquid load for one hour.

STEP 7: POTASSIUM CARBONATE (5ml) AND BENZENE (2ml)

Evaporated tubes occasionally occur during heating and are easily identified by dark colored tubes and no remaining liquid. If any tubes have evaporated, they will no longer be of any use... discard. Tubes generally evaporate if the seal between the cap and tube is not flush, or if they were not capped tightly.
Wipe off spigots of K₂CO₃ and Benzene bottles with Chlor-Meth. Remove bubbles from repipet lines before carefully adding reagents to tubes. Keep the caps in order. Remember not to cross-contaminate samples by touching tip to test tubes. Again, it is best to add to samples first, then each set of blanks, and standards, recapping tubes in between.

Vortex on highest setting until uniformly mixed. Should look milky.

**STEP 8: CENTRIFUGE (3000 rpm)**

Fill centrifuge buckets (red sleeves) with test tubes. Fill empty spaces with empty, capped tubes as well. This will help keep sample tubes from rattling and breaking. Leave one empty tube in each bucket uncapped so that you can add water to balance. Be sure to balance the caps also.

Set centrifuge to 11 min. Brake at 50%. Speed at zero. Turn on and slowly increase speed to 3000 rpm, to further avoid breaking tubes. It should take about one minute to bring it up to speed.

**STEP 9: ASPIRATING**

Label mass spec vials being careful not to touch tops even while wearing gloves. This can be done while centrifuging. Rinse capping gun.

The centrifuge process has brought free fatty acids up in the yellow-tinted top layer (benzene). They should be uniform throughout the layer. Use a new pipet for each sample retrieved, and aspirate from center of top layer, being careful not to touch the lower layers with the pipet tip. It is best to hold the test tube between thumb and forefinger, and the mass spec vial between forefinger and long finger. Then, aspirating with free hand can be done away from open samples (in case a drop or two escapes). If you attempt to pipet into mass spec vials directly into rack, a spill could contaminate other samples. Vials 3/4 full are ideal.

After completing aspiration, cap all mass spec vials and freeze FFA samples (in the rack) in cold freezer.

**STEP 10: ASPIRATE AND DRY SAMPLES**

Preheat block to 65°C. Aspirate as in step 9 with unbroken pipets. Use one pipet for meat samples, discard, and then one for each set of different standards.

Pour carefully into clean, unused, labelled duran vials. Place duran vials on block and dry. Be sure your samples are completely dried.

Samples can be stored at this point if capped and stored at room temp. Be sure to cap tightly.
STEP 11: DERIVATIZING SAMPLES: ACN (100μl) & BSTFA (100μl)

Do not continue unless mass spec is ready!! Preheat block drier to 65°C.

Use Hamilton syringes labelled specifically for ACN and BSTFA. The BSTFA syringe often becomes clogged (crystallizes) and can be cleaned with Methanol. If this happens, allow at least 30 min. drying time for Methanol to completely evaporate from inside the syringe, and then rinse by drawing small amount of BSTFA and flushing before using. Squirt two shots of each derivatizing agent into duram vials, cap tightly, vortex for 3-4 sec., and heat for one hour on block at 65°C. Be careful not to touch vials with Hamilton.

While derivatizing agents are handy, prepare 3-4 washes (8 washes for assays >60 tubes) for the mass spec. Add two shots each of ACN and BSTFA to mass spec vials, cap, label and vortex.

STEP 12: MASS SPEC VIALS

After samples are heated, transfer into labelled mass spec vials as in step 11. Use a new pipet for each sample. Remember to rinse capping gun before capping samples.

STEP 13: GCMS ANALYSIS:

Analyze the prepared samples on the GCMS using the method, DIR:CHOLSIM.M, which has been set-up to monitor the samples for specific cholesterol ions.

7.0 MAJOR HAZARDS INVOLVED:

7.1 Hazards: See MSD Sheets for more information
   7.1.1 ACETYL CHLORIDE (reacts violently with water)
   7.1.2 CHLOROFORM, METHANOL, BENZENE, ACETYL CHLORIDE, ACN, BSTFA

7.2 Protective equipment required:
   GLOVES: Y
   CLOTHING: Y
   EYE PROTECTION: Y
   HOOD: Y

8.0 Quality Control:
   Described in detail in procedure section
   ** Always wear latex gloves!!

9.0 Disposal of materials:
9.1 Biological materials:
   9.1.1 Sink: Y
   Garbage: Y
   Meat Laboratory: N
   Other: Y (Organic waste container)

SOP # 118, C:\SOP\Cholanal
Page 6
Cholesterol Standard
Storage: small flask, tightly sealed, frozen
Storage location: -20°C, white freezer, Rm. 329
Shelf life: 4 months
Special needs: none
Preparation: 100 mg cholesterol crystals (??)/100 ml propanol

alternative Cholesterol Standard
Storage: small flask, tightly sealed, frozen
Storage location: -20°C, white freezer, Rm. 329
Shelf life: 4 months
Special needs: none
Preparation: 44.44 ml Oxford commercially-prepared standard (450 mg/dl)/100 ml Oxford diluent

Fatty Acid Standard
Storage: small flask, tightly sealed, frozen
Storage location: -20°C, white freezer, Rm. 329
Shelf life: 4 months
Special needs: none
Preparation: 100 ul concentrate in 25 ml propanol

12.0 DATA FORMS/CALCULATION: none
ASSAY RECORD
SOP#118, Cholesterol, Free fatty acid transesterification

Date: ____________________ Personnel Signature: ________________
Sample ID: __________________________________________________________________________

# Samples: __________________ Scale Calibration (if used): ______________

Pipetman test, if needed: __________________________________________________________________

Reagents Used: Chloroform/Methanol - date: ___________
Methanol/Benzene - date: ___________
6% K.CO₃ - LOT#: ___________
Internal Standard - date: ___________
Cholesterol Standard - date: ___________
Fatty Acid Standard - date: ___________

Analysis: GCMS start date: ____________________
end date: ____________________

Assay Notes:
APPENDIX IV. STATISTICAL PROCEDURES
DATA A; infile in;
    input trt $ id btemp itemp marb rfat rchol rawm rawp cfat cmoist
cprot cchol a b c d e f g h
tend juicy fides flint ovpal;
    cards;
NOTE: The infile IN is:
    Osname=A1$JCI.FATTEXT,
    Unit=3380, Volume=PUB007, Disp=SHR, Blksz=6345,
    Lrec=235, Recfm=FB
NOTE: 168 records were read from the infile IN.
NOTE: The data set WORK.A has 168 observations and 26 variables.
NOTE: The DATA statement used 0.23 CPU seconds and 147K.

proc sort;
    by trt;
NOTE: SAS sort was used.
NOTE: The data set WORK.A has 168 observations and 26 variables.
NOTE: The PROCEDURE SORT used 0.06 CPU seconds and 1500K.

proc glm;
    class trt btemp itemp;
    model cfat cmoist cprot cchol a b c d e f g h
tend juicy fides flint ovpal
    = trt btemp itemp trt*btemp trt*itemp trt*btemp*itemp
    btemp*itemp;

The SAS System
General Linear Models Procedure
Class Level Information

Class   Levels   Values
  TRT    6       C H L P O S
  BTEMP   2       1 2
  ITEMP   2       1 2

Number of observations in data set = 168

Group  Obs  Dependent Variables
  1    157  CFAT
  2    156  CMOIST CPROT
  3    164  CCHOL
  4    160  A B C E F G H
  5    161  D
  6    168  TEND JUICY FIDES FLINT OVPAL
General Linear Model Procedure

Dependent Variable: CCHOL

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NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.
General Linear Models Procedure

T tests (LSD) for variable: CCHOL

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha = 0.05  df = 140  MSE = 48.01219
Critical Value of T = 1.98
Least Significant Difference = 2.1396
Harmonic Mean of cell sizes = 81.9878

Means with the same letter are not significantly different.

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General Linear Models Procedure

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<p>| BTEMP | ITEMP | CCHOL LSMEAN | Std Err | Pr &gt; |T| | Pr &gt; |T| HO:LSMEAN(i)=LSMEAN(j) |
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| 1     | 1     | 63.1380952   | 1.06918017 | 0.0001 | 1  | 0.0771 | 0.9160 0.0586 |
| 1     | 2     | 65.8494048   | 1.0839287  | 0.0001 | 2  | 0.0884 | 0.8913 0.0757 |
| 2     | 1     | 63.2990079   | 1.0839287  | 0.0001 | 3  | 0.0884 | 0.8913 0.0757 |
| 2     | 2     | 66.0608746   | 1.0984788  | 0.0001 | 4  | 0.0586 | 0.8913 0.0757 |</p>
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The SAS System

General Linear Models Procedure
Least Squares Means

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The SAS System

General Linear Models Procedure
Least Squares Means