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I am submitting herewith a thesis written by Helen Virginia Gann entitled "Feeding Management Effect on Flavor and Selected Chemical Characteristics of Frozen Ground Beef." I recommend that it be accepted in partial fulfillment of the requirements for a Master of Science Degree, with a major in Food Technology and Science.

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FEEDING MANAGEMENT EFFECT ON FLAVOR AND SELECTED CHEMICAL CHARACTERISTICS OF FROZEN GROUND BEEF

A Thesis

Presented for the

Master of Science

Degree

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Helen Virginia Gann

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ABSTRACT

Thirty-nine steers were divided into thirteen trios according to breed, body type and weight. One of each trio was finished on pasture (orchard grass and clover, low energy ration), another of each trio was finished on grain but limited (low energy ration) to gain weight at the same rate as pasture-finished, and the other of each trio was finished on unlimited grain (high energy ration). Flavor and selected chemical characteristics of ground beef prepared from the semimembranosis muscle and fat trim of the brisket from the left side of each carcass were determined after 30 and 180 days storage at -18°C. A selected panel of six members consistently scored ground beef from steers finished on pasture approximately 1.3 to 1.4 units lower on a 7 point scale (1 = dislike extremely more than reference to 7 = like extremely more than reference) than ground beef from steers finished on a limited or full grain ration. No differences in flavor scores were found for storage.

Ground beef from pasture-finished steers had significantly less free sugars than ground beef from grain-finished steers. Ground beef from low energy ration finished steers had significantly higher free fatty acids and lower quantities of lipid oxidation products than ground beef from high energy ration finished steers. All chemical characteristics (free fatty acids, lipid oxidation products, free amino compounds and free sugars) increased significantly during storage. Fatty acid analysis of neutral lipids and polar lipids of

ground beef from steers of Angus breed only (18 animals) showed that pasture finished steers had less palmitic and more stearic and linolenic acid in both neutral and polar lipids than steers finished on limited grain. Ground beef from steers finished on low energy rations had more myristoleic, palmitoleic and stearic acid and less linoleic and linolenic in neutral and polar lipids and more palmitic acid in the neutral lipids than ground beef from steers finished on high energy ration.

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CHAPTER I

INTRODUCTION

Beef consumers in the United States are beginning to eat more meat from lower cost, grass-fed (low energy ration) cattle than previously had been eaten before. There have been complaints, however, of off-flavor in meat from grass-fed cattle, especially after frozen storage.

Desirable beef flavor is difficult to subjectively evaluate and many factors have been shown to affect it. It has not been established whether ration or feeding management has a significant influence on flavor of frozen beef. Some studies have shown that flavor of meat from pasture-fed steers is consistently rated lower than that from steers which had received some grain, yet others have found no significant differences. The water soluble non-protein nitrogen (NPN) and water soluble carbohydrates in the lean have been shown to be in part responsible for development of flavor during cooking. However, the NPN and water-soluble carbohydrate content of beef fed different rations have not been studied to determine if the flavor differences arise from these components. Lipid portions of meat have been shown to contain the flavor components that are associated with the development of flavor of different species, i.e. beef, pork and lamb. There is some question as to whether the intramuscular fat or the external fat contribute to the desirable flavor

complex of cooked beef. Perhaps only particular fat fractions are responsible for good beef flavor.

Lipid degradation has been associated with the development of off-flavors in meat; thus the development of off-flavor in frozen grass-fed beef could be due to deterioration of lipid fractions. It has been shown that free fatty acid content of beef increases with frozen storage time, primarily due to phospholipid hydrolysis. Also, frozen meat becomes rancid because of lipid oxidation at different rates depending upon several factors: freezer temperature, muscle type, unsaturation of fatty acids and phospholipid content.

Previous research has investigated the effects of different rations as well as the effects of different energy levels on flavor and some related chemical parameters of beef frozen at different temperatures for various lengths of time. However, there seems to be a lack of information concerning beef finished on grass alone compared with beef finished on low energy grain rations and high energy grain rations. This lack is also found in the investigation of ration effects on the combination of flavor and chemical parameters of beef frozen up to six months. The purpose of this investigation was, therefore, to study the effects of ration and ration energy level on flavor and certain chemical parameters of ground beef after 30 days and 180 days frozen storage. The chemical parameters investigated were total free fatty acids, thiobarbituric acid values, water-soluble NPN compounds, water-soluble carbohydrate content, total lipids and fatty acid composition of polar and neutral lipid fractions.

CHAPTER II

REVIEW OF LITERATURE

I. FATTY ACID COMPOSITION OF BEEF

Efforts have been made to study two types of animal lipids: phospholipids primarily from muscle tissue, and neutral lipids primarily adipose tissue. Horstein et al. (1961) fractionated beef lipids, determined the fatty acid composition of the phospholipid fraction and neutral lipid fraction, and studied the possible effect of phospholipids on meat flavor. Polyunsaturated fatty acids made up 10% of the neutral fraction and 50% of the phospholipid fraction.

The questions have not been completely resolved as to whether there is a significant difference in fatty acid composition of beef lipid due to type of feed or level of energy allowed the ruminant. Also, it has not been reported whether fatty acid composition affects palatability.

Fatty acid composition varies with location (O'Keefe et al., 1968; El Gharbawi and Dugan, 1965; Horstein et al., 1967; Terrell et al., 1967; Marchello et al., 1968; Keller and Kinsella, 1973). Marchello, Dryden and Ray (1968) in comparing the fatty acid content of three muscles found that the semimembranosis (SM) had a lower percent saturated fatty acids (49.82%) than triceps brachii (TB) (50.41%) or longissimus (LD) (52.91%). The SM also had a higher level of unsaturates (50.17%) than TB (49.53%) or LD (47.13%).

Fat present in beef carcasses is synthesized in the body from carbohydrates and protein. Since fatty acids in the diet are partially or completely hydrogenated by rumen microorganisms (Garton, 1960), the degree of unsaturation of body fats in cattle is affected little by diet. Reiser and Reddy (1956) found larger amounts of stearic acid in depot fat than the diet provided, supporting the belief that diet effects in cattle are not always predictable.

There is conflicting information of the effects of diet on fatty acid composition. Privett et al. (1965) reported in an early study that type of diet affected the fatty acid composition of depot fats. Shoulders and rounds of beef cattle fed high energy diets contained lower percents of saturated fatty acids (and greater amounts of oleic and linoleic acids) than beef cattle fed a low-level maintenance diet. Edwards et al. (1961), in an experiment studying dietary fat in animal rations, found that addition of animal fat to steer rations resulted in a highly significant increase in stearic acid content of rib fat. In another part of their experiment using samples of rib fat of 20 pasture steers whose ration had been supplemented with two types of corn (corn + 10% animal fat and corn + salt) data showed a greater deposition of myristic acid in steers receiving corn with 10% animal fat added.

Cook (1963) found no effect of feeding management and aging time (grass finished vs grain finished) on the neutral fraction of raw and cooked beef lipids. In the phospholipid fraction, three unidentified components were noted that increased in concentration with aging time

and these were of greater percentages in fat from grass fed than grain fed cattle.

Cabezas et al. (1965) studied the effect of physical form of diet and the ratio of dried citrus meal to corn meal on composition of fatty acids in beef and found a diet effect on fat composition. Data on rib fat composition revealed that diets containing 72% corn produced a higher ($P<0.05$) degree of unsaturation than diets containing partial fiber replacements of corn. These changes were associated with higher starch content in corn diet. Church et al. (1967) found differences in the percent fatty acids of depot fat of cattle fed wheat vs barley as they experimented with the addition of tallow fat. Addition of tallow resulted in significant increases in $C_{14:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, and a decrease of $C_{18:1}$. Clemens et al. (1973) in a study of 144 Angus calves, half fed high energy and the other half a low energy diet, found stearic acid to be the only fatty acid influenced by dietary energy level. There was a higher amount in low energy fed fat.

However, in a study of 156 Angus and Polled Hereford steers in six treatments of varying diets of cracked soybeans, Skelley et al. (1973) found fatty acid composition varied between animals, and feeding treatments had little effect on composition of fat. Sumida et al. (1972) found a feeding treatment effect on fatty acid composition, yet the treatments did not affect sample sites in the same manner. Feedlot steers had higher levels of myristic acid and stearic acid. Outer subcutaneous depot fat showed higher oleic acid in feedlot steers, but the same fat in pasture steers had higher stearic acid levels.

Grass-fed animals tend to deposit a yellow tinged fat due to the transfer of carotene from pasture to body fat (Bull et al., 1941; Wanderstock and Miller, 1948; Malphrus, 1961). Malphrus (1961) presented carcass data on steers full fed drylot or pasture and noted pasture fed steers' fat (2.8) was more yellow than drylot steers (4.0) (4 point scale: 4 = white, 2 = moderately yellow). Pasture fed steers had a higher amount of carotene in carcass fat (pasture, 1.82 ug/g fat and drylot, 1.13).

Information is sketchy concerning the relationship of fatty acid composition to muscle palatability. Younathan and Watts (1960) and Hornstein and Crowe (1960) suggested the neutral lipid fraction may enhance flavor and phospholipids may have a negative effect on muscle flavor. Dryden and Marchello (1970) found significant relationships between certain fatty acids in longissimus lipid and taste panel scores. Yet, Waldman et al. (1965), Terrell et al. (1967) and Skelley et al. (1973) found that fatty acid composition is not related to palatability factors.

II. LIPID DETERIORATION DURING STORAGE

Deterioration of lipids in meat has been shown to cause off-flavors during storage (Lea, 1957; Younathan and Watts, 1960; Hornstein et al., 1961; Pearson, 1968). In cold storage, even at -20°C, there is a slow uptake of oxygen by fatty tissues which form peroxides. Upon thawing, aldehydes and ketones form, leading to oxidative rancidity (Callow, 1952). Lipolysis of phospholipids during frozen

storage was observed in vobine muscle and resulted in rancidity (Lea, 1957; Awad et al., 1968; Greene, 1969). The rate of oxidative change is affected by many factors. The more unsaturated the fat is, the faster it will oxidize. Fat from cattle fed low level diets is more susceptible to oxidation than fat from cattle fed high level diets. Young animals' fat is more susceptible to oxidation than older animals' fat. (As meat storage time increases, oxidation of fat increases.) (Lea, 1958).

Neutral lipids, which compose about 95% of total lipids in beef, oxidize slower than phospholipids (Lea, 1957; Watts, 1962, El Gharbawi et al., 1965). The study by Edwards et al. (1961) necessitated reserving fat samples for 16 months at 0°F while chemical methods were being developed. To ascertain whether fatty acids had changed during storage, fat samples from three similarly treated, freshly killed steers were analyzed. Chromatograms exhibited the same pattern of peaks, supporting Privett et al. (1955) who found that fatty acids of beef fat undergo no appreciable oxidative deterioration during low temperature storage. Privett et al. (1955) noted that dietary factors played a negligible role in the overall destruction of unsaturated fatty acids in whole cuts of beef during storage, but diet influenced the rate of deterioration of surface fat.

The 2-thiobarbituric acid (TBA) method of Tarladgis et al. (1960) is a widely used test for determining oxidative rancidity. Malonaldehyde, which is an end product in the oxidative decomposition of fats, reacts with TBA to give an empirical measure of the deterioration of

fatty tissue. Pearson (1968) determined TBA values on fresh meat and meat stored at 5°C, periodically, up to 20 days. TBA values of meat with 11.2% fat increased from 0.02 mg malonaldehyde/kg meat at 0 day to 2.05 at 20 days, and TBA values of fatty tissue meat with 70.2% fat increased from 0.38 to 0.72. These values support the theory that the Tarladgis (1960) method assesses rancidity in neutral fats as well as phospholipids. High TBA values in lean beef were probably due to high phospholipid content.

Keskinel, Ayres and Snyder (1964) reported an increase in TBA numbers (mg malonaldehyde/kg meat) of ground beef frozen 14 1/2 months, thawed and then refrigerated up to three weeks with TBA of samples being measured during the refrigerator storage. TBA numbers were 0.52 at one day refrigeration and 7.21 after 10 days refrigeration. Keskinel et al. (1964) and Terrell et al. (1967) reported no changes in fatty acid content during freezer storage. Awad, Powrie and Fennema (1968) followed a progressive rise in TBA number (TBA number was equal to optical density) during an eight week (-4°C) storage on beef round muscle: 0.02 in fresh beef to 0.27 at eight weeks frozen storage. Keller and Kinsella (1973) reported an increase in TBA numbers (mg malonaldehyde / kg meat) of ground chuck (from 0.35 before freezing to 0.88 at 16 weeks at -18°C) and ground beef (0.53 before freezing to 1.46 at 16 weeks frozen storage at -18°C). However, ground round TBA numbers decreased slightly (0.49 to 0.35) indicating some oxidation occurred in neutral fats and none in the more polar fats of the ground round. Jakobsson and Bengtsson (1973) also found a significant increase in frozen ground beef TBA values (measured as extinction at

530 nm/g fat) from one month (19.8) to nine months (27.6) along with a general decrease in quality assessed by flavor scores. No studies were found which investigated the effect of ration on TBA values and subsequent changes during storage.

Another characteristic contributing to deterioration of meat fat quality is the increase in free fatty acids (FFA). This increase is due to hydrolysis of the lipid, perhaps due to enzyme activity (Lea, 1960). Lea (1960) reported that free fatty acids liberated by the hydrolysis of animal fats do not have a serious effect on flavor and the tainted off-flavors which develop are probably due to protein decomposition which may cause off-flavors to accumulate in the fat. In an early study Lea (1931) reported an increase in free fatty acids in ground beef which coincided with the development of unpleasant flavors in cooked meat. FFA was 1.5% (expressed as percent oleic acid in fat) at 25 days of 0°C storage and 5-11% at 42 days storage. The unpleasant flavor was noticed at 2-3%.

Broumand, Ball and Stier (1958) reported a progressive increase in free fatty acids of ground beef during storage at 40°F from 1.6 to 6.7%. Rockwood, Ramsbottom and Mehlenbacher (1947) utilized chloroform extraction of animal tissue fats for determining free fatty acids on the principle that the fat is not altered in free fatty acid content during extraction and that the fatty acids (weak) can be titrated with an alkalai of known normality. Pearson (1968) in a study of stored beef (5°C) found an increase in free fatty acids from 0.24% in fresh meat to 1.77% in meat described as "just spoiling."

(This was expressed as percent free oleic acid in fat.) In identifying points where meat is spoiled, maximum acceptability limit of 1.8% oleic acid was recommended.

III. WATER SOLUBLE COMPONENTS OF BEEF

Certain precursors of cooked beef flavor are extractable with cold water (Hornstein et al., 1960, and Batzer et al., 1960). The water soluble components of meat have been of interest to researchers for a variety of reasons. These components which include free amino acids and free carbohydrates are important as flavor and odor precursors which develop cooked meat flavors upon heating (Macy, 1964a and b; Wasserman and Gray, 1965). Study of these water soluble components has also been conducted to investigate their association with differences in quality of meat (Locker, 1960; Saffle et al., 1961; Doty and Pierce, 1961; Matlock and Hiner, 1961; Gardner and Stewart, 1966; Jarboe and Mabrouk, 1974).

Research has been conducted to study proteolysis of meat during refrigerator storage attributed to microbial and enzymatic activity (Locker, 1960; Saffle et al., 1961; Gardner and Stewart, 1966). Saffle et al. (1961) theorized that alpha amino groups could be released as bacteria broke down muscle protein. Ninhydrin is a reagent that forms blue in the presence of alpha amino groups; thus, changes in the amount of (free) alpha amino groups would give a more intense blue in the presence of ninhydrin. Saffle et al. (1961) found that ninhydrin optical density increased (from 0.035 to 0.224) at 635 nm

as meat quality decreased; their method utilized a water extract, but the method did not deproteinate the extract. Locker (1960), however, studied protein breakdown of meat by measuring non-protein nitrogen by a Kjeldahl method and ninhydrin reactive free amino groups of deproteinated water extractions. Free amino acids were 53 μmoles/g muscle initially and 68 after 14 days at 2°C in a study of ox *longissimus* muscle. Non-protein nitrogen increased over 14 days (from 274 μmoles/g muscle to 297). Increases were attributed to aminopeptidases breaking off terminal nitrogen groups of proteins.

In 1961, Doty and Pierce studied changes in percent of free amino nitrogen in raw meat using a gasometric amino nitrogen apparatus. Raw meat contained 0.05-.08% free amino nitrogen, which was 1-2% of the total nitrogen present. Aging time increased the percentage free amino nitrogen which was most likely due to autolysis. This increase was considered an important flavor change since only small amounts of simple peptides are required for detectable flavor.

Ma *et al*. (1961) used paper chromatography developed with ninhydrin reagent to study variations in free amino acid content of beef muscles. They found increased amounts of free amino acids (leucine and isoleucine) when cuts were more tender. Gardner and Stewart (1966) studied changes in the levels of 21 free amino acids by electrochromatography during refrigerator storage of cow meat. A total of 670 μg free amino acids/g meat (wet weight) was partitioned into identifiable amino acids and their changes followed. Increased "free amino acids" were attributed to microbial growth, temperature, and

length of storage time. Macy (1962a) noted the free amino acid content of beef, lamb, pork and fish were similar. Variation of free amino acid content has not been reported on beef fed different rations, and reports of changes over frozen storage were not found in the literature.

Another class of water soluble flavor precursors that are important in beef, even though they make up less than 1% total, are the carbohydrates. A number of studies have been conducted to identify sugars in beef and the role they play in browning reactions and flavor development (Horstein and Crowe, 1960; Macy et al., 1964a and b; Pearson et al., 1966; Wasserman and Gray, 1965; Mabrouk et al., 1969; Jarboe and Mabrouk, 1974). Most of this research is involved with identification of individual sugars. Jarboe and Mabrouk (1974) identified the free sugars as alpha glucose, beta glucose, fructose, inositol, and ribose. Ingram and Dainty (1971) found a decrease in carbohydrate concentration with an increase in bacterial level in meat during refrigerator storage. However, no reports of research were found which examined changes in free sugars or water soluble carbohydrate content during frozen storage.

Water soluble components, in general, have not been studied during freezer storage. Freezing of meat retards growth and activity of microorganisms and the enzymatic and chemical reactions that cause deterioration and spoiling, yet these elements are in meat and to date neither the changes in water soluble components during freezing nor resulting flavor changes have been investigated.

IV. ACCEPTABILITY OF GRASS-FED BEEF

Research has shown that grass-fed beef does not have the flavor or grain fed beef. Bull et al. (1941) reported on numerous studies that indicated consistently lower panel ratings for forage fed beef. Wanderstock and Miller (1948) showed significantly lower taste panel scores for pasture fed beef than grain fed. Jacobson and Fenton (1956) concluded that beef animals on a high energy level of nutrition high higher flavor scores than animals on medium and low energy levels. Meyer et al. (1960) reported higher sensory ratings on flavor of grain fed beef when compared to grass fed in an aging study. Bond et al. (1972) found that meat from steers fed a high percentage of concentrate was more desirable to taste panels, followed by meat from cattle fattened on hay with a small amount of concentrate prior to slaughter. Least desirable was meat from steers fattened on hay diets alone. No studies were found in the literature examining the frozen storage effect on acceptability of grass fed and grain fed beef.

CHAPTER III

MATERIALS AND METHODS

Thirty-nine steers weighing 460-680 pounds were grouped into trios (13) on the basis of breed, weight and body type similarities. All steers were fed a silage ration from October, 1975, through April 14, 1976. At that time, one of each trio was randomly assigned to one of three feeding treatments, 13 animals to each treatment, in a completely randomized block experimental design. Treatment 1 for this study consisted of unlimited grazing (Ad libitum) on a pasture of orchard grass and fescue. Treatment 2 was placed in a dry lot and fed a grain ratio to allow growth of 1.4-1.5 pounds per day. (This is equivalent to standard values for spring pasture gains.) Treatment 3 was assigned to a dry lot and allowed to consume an unlimited amount of grain (Ad libitum). Subsequent references to the treatments will be treatment 1 as pasture, treatment 2 as limited fed and treatment 3 as full fed.

The cattle were slaughtered at the University of Tennessee abbatoir on three dates during the first two weeks in August, 1976. Trios were slaughtered and analyzed simultaneously to remove all external effects so that differences within the trios were due solely to the feeding treatment. All carcasses were chilled for 72 hours at 1.6°C and then fabricated into the wholesale cuts.

For this investigation, ground beef was prepared from the semimembranosis muscle of the left side of each carcass and the external fat of the left brisket in the following way: the lean muscle was ground two times and the fat trim once through a one-half inch plate of a Hobart Model 4722 meat grinder. The modified Babcock fat analysis (Ockerman, 1969) was used to approximate the fat content of the ground muscle and fat trim. Ground beef was formulated to contain approximately 20% fat by Sausage Square (Terrell, 1971). The combined fat trim and lean was then ground through a one-eighth inch plate. After grinding, portions of each ground beef sample were placed in Whirlpak bags and saved until analyzed for protein nitrogen (AOAC, 1970) and moisture (AOAC, 1970). The rest of the ground beef from each steer was packaged in 1000 g portions and wrapped in freezer paper. The packages were frozen overnight at -34°C. and stored at -4°C. until analysis at 30 days or 180 days.

A 9 cm square, approximately 0.65 cm thick, of brisket fat was obtained for evaluation of color with the Munsell Color Tabs and determination of Hunter color values L, a, and b, by the Hunterlab Color/Difference Meter D2502 which was calibrated with the white vitrolite tile: L = 93.4, a = 1.10, b = 1.90. Samples were read on both the inside and outside of the 9 cm fat square. Carotenoid pigments were extracted from the brisket fat and total carotenoid determined (Kirton et al., 1975).

I. CHEMICAL ANALYSIS

At each storage time (30 days and 180 days) a 1000 g portion of ground beef from each steer was taken from the freezer and allowed to thaw at room temperature for seven hours. At this time, samples from each steer were weighed into Whirlpak bags in the weight required for each of the following chemical analyses: Thiobarbituric acid test, ninhydrin reactive materials, carbohydrate content, lipid extractions for free fatty acid determination, and lipid extraction for fatty acid composition determination. The samples were flushed with nitrogen, sealed and stored at -4°C for analysis within one week.

Thiobarbituric Acid Test

The distillation method of Tarladgis et al. (1960) was used for a quantitative determination of end products of lipid oxidation in the ground beef by measuring the absorbance of the reaction products of 2-thiobarbituric acid (TBA) with oxidation products at 538 nm.

A standard curve was prepared by determining the absorbance at 538 nm of solutions containing 1×10^{-9} to 7×10^{-8} moles of malonaldehyde per 5 ml after the malonaldehyde had been reacted with TBA reagent. This curve was used for determining the relative amount of lipid oxidation products in the distillate from ground beef. The regression coefficient for all standard curves was 0.9976 and the equation was:

$$\text{Malonaldehyde } (10^{-8} \text{ moles}) = 9.3122 \text{ (Absorbance)} - 0.4638.$$

Absorbance values for the TBA complex with oxidation products.

of ground beef were converted to mg malonaldehyde per 1000 g of ground beef and referred to as "TBA numbers."

The following solutions were used in this procedure:

1. 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid, dissolved by heating in a boiling water bath.
2. 1,1,3,3-tetra-ethoxypropane in deionized water, stock solution of 1×10^{-3} M.
3. 4 N HCl Solution: one part (35-36%) hydrochloric acid to two parts deionized water.

Ten g of ground beef were blended two minutes with 50 ml of deionized water in a Virtis Homogenizer at high speed. Hydrochloric acid solution (4N) was added to bring the solution to a pH of 1.5 (approximately 2.5 ml); the mixture was quantitatively transferred by 47.5 ml of deionized water to a Kjeldahl flask (total volume of extraction fluid 100 ml). Boiling beads and 0.5 ml of Dow Antifoam A were added to prevent foaming and bumping. Flasks were placed on the Kjeldahl distillation rack and heated at highest setting. Fifty ml distillate was collected in graduated cylinders in 10 to 15 min. During heating, the flasks were mixed occasionally to prevent burning of the groun beef mixture.

Two extractions were done for each sample, and duplicate 5 ml portions of each 50 ml distillate were pipetted into test tubes and mixed with 5 ml of 0.02 M TBA reagent Marbles were placed on the test tubes to act as condensers, and the tubes were placed in a boiling water bath for 35 min. A blank was prepared by reacting 5 ml of

deionized water with 5 ml TBA reagent. The test tubes were cooled in tap water for 10 min. Portions of the reaction mixture were transferred to a cuvette and absorbance values were determined at 538 nm on a Coleman Hitachi Model 124 Double Beam Spectrophotometer.

Ninhydrin Reactive Materials

The method of Piotrowski et al. (1970) was modified to produce a water extract of ground beef which was used for the determination of ninhydrin reactive materials (Mickelson, 1969) and also for determination of simple carbohydrates (DuBois, 1956).

Ten g of ground beef were blended with 100 ml of cold water for 5 min. The mixture was centrifuged at 5°C and 6000 rpm for 10 min. The supernatent was separated from the insoluble residue and filtered through glass wool to remove any fat particles. Fifty ml were then deproteinated with 3 g trichloroacetic acid for 30 min and centrifuged 10 min (5°C at 6000 rpm).

Water soluble ninhydrin reactive material (NRM) was calculated in units of mg glycine equivalents. A standard curve for the concentration range of 5×10^{-3} to 2.5×10^{-2} mg glycine/ml was determined. The regression coefficient for all standard curves was 0.9980 and the equation was:

$$\text{NRM (mg glycine/ml)} = 422.4134\ (\text{Absorbance}) + 1.9018.$$

The following solutions were used:

1. Ninhydrin reagent: 95 g KH_2PO_4, 43 g anhydrous Na_2HPO_4, 5 g triketohydrindene hydrate, and 3 g fructose made up to 1 liter with deionized water.

2. Ninhydrin diluent: 2 g KIO_3, 400 ml 95% ethanol, and 600 ml deionized water.

One ml of the deproteinated extract was diluted to 10 ml and 1 ml of this was mixed with 1 ml ninhydrin reagent and held in a boiling water bath for 15 min. After cooling, 5 ml ninhydrin diluent were added and absorbance read at 570 nm on a Coleman Hitashi Model 124 spectrophotometer against a blank composed of 1 ml water treated like the deproteinated extract.

Carbohydrate Content

Total carbohydrate content of the water extract was determined by the phenol sulfuric acid method of DuBois et al. (1956).

One ml of the deproteinated water extract was diluted to 10 ml and 2 ml of the dilution pipetted into 10 ml test tubes. Five ml of concentrated sulfuric acid and 1 ml of 5% phenol solution were added and the sample mixed well. The test tubes were held at room temperature for 10 min and then placed in a 25°C water bath for 10 min. Absorbance was read at 480 nm on the Coleman Hitashi Model 124 Spectrophotometer with the reference sample composed of 2 ml of deionized water reacted like the samples.

A standard curve was established for glucose solutions from 2 x 10^{-5} to 14 x 10^{-5} µg/L. The linear regression coefficient was .9894 for all standard curves and the equation of the standard curve was:

µg glucose/L solution = 151.52 (Absorbance) -7.48.

Lipid Extractions

Ground beef lipids were extracted and quantitated by a modified procedure of Ostrander and Dugan (1961). Forty g of ground beef were blended with 130 ml reagent grade methanol for 5 min at speed 40 on a Rheostat. Sixty-five ml of chloroform were poured down the sides of the blender jar and the mixture reblended for 5 min. An additional sixty-five ml chloroform were added and blended for 20 sec and 1.5 g of zinc acetate dissolved in 65 ml of deionized water was added and blended for 10 more sec. This mixture was filtered under a stream of nitrogen through Whatman No. 1 filter paper in a Buchner funnel with suction. The filtrate, filtrate paper and one half tissue were reblended for 2 1/2 min with 100 ml of chloroform. This mixture was filtered again through filter paper into the same Buchner funnel and suction flask. The blender jar was rinsed with 75 ml chloroform which also was filtered into the chloroform extract. After filtering was complete, all of the filtrate was poured into a 500 ml graduate cylinder and the suction flask rinsed with 25 ml of methanol.

The extraction was placed in a -4°C freezer until a sharp interface was apparent between the methanol layer and the chloroform. The volume of the chloroform layer was recorded. The solution was then poured into a 500 ml separatory funnel and upon resolution of the two layers, the bottom layer was drained off, flushed with nitrogen and reserved for future analysis. Percent lipid was quantitated by pipetting 5 ml portions of the chloroform solution into pre-weighed 50 ml beakers. The solution was allowed to evaporate overnight, and

the beaker and solid residue was dried in a vacuum oven at 75°C for 1 hour, cooled in a dessicator and re-weighed.

$$\text{percent lipid} = \frac{\text{ml } CHCL_3 \text{ layer} \times \frac{\text{g fat}}{5 \text{ ml}} \times 100}{40 \text{ g meat extracted}} .$$

Two ground beef extractions were prepared for each sample at each storage time. One extraction was used for free fatty acid determination and the other extraction was used for silicic acid column fractionation and subsequent fatty acid composition analysis of the two lipid fractions.

Free Fatty Acids

The method of Rockwood et al. (1947) was used for free fatty acid determination. Fifty ml of the chloroform extract was pipetted into a ground glass stoppered Erlenmeyer flask in duplicate for each extraction. A the same time, 5 ml of the extract was pipetted into dried pre-weighed 50 ml beakers to determine the weight of fat in the 50 ml portion. The 50 ml sample was concentrated to dryness on a rotary evaporator at 40°C. During this time 50 ml of 99% ethanol was mixed with 1 ml of 1% phenolphthalein indicator in ethanol and neutralized by adding 0.1 N NaOH until the ethanol was permenently light pink. Fifty ml of neutralized ethanol was added to the Erlenmeyer containing the fat residue and the flask placed in a warm water bath (55°C). The ethanol-fat while being stirred by a magnet and magnetic stirrer was titrated with 0.1 N NaOH. The volume required for a pink color lasting one min was recorded and free fatty acids calculated as percent

free oleic acid:

$$\frac{(\text{Normality of NaOH})\ (\text{ml NaOH})}{\text{weight fat in 50 ml}} \times \frac{282.85\ (F.W._{oleic})}{1000} \times 100.$$

Fatty Acid Composition

Due to the large number of samples in this study, it was decided to study the fatty acid composition of only six trios (18 animals) all Angus breed. The second lipid extraction was concentrated to less than 10 ml volume on the rotary evaporator at 40°C. It was then made up to volume quantitatively in a 50 ml volumetric flask, with 20:3 (v:v) chloroform:methanol solution. The amount of lipid (g/ml) was determined, and approximately 1 g of lipid was subjected to silicic acid column separation.

Silicic Acid Column Chromatography

The beef lipid extractions were separated into polar and neutral fat fractions by the silicic acid column chromatographic method of Horstein et al. (1961). Fifty g of Mallinckrodt AR 100 mesh silicic acid was heated overnight at 130°C, cooled in a desiccator and slurried with 100 ml of 3:1 chloroform:methanol for 30 min. Forty ml of the fluid was decanted and discarded, and the slurry poured into a 3.0 x 108 cm glass column fitted with stopcock and sintered glass disc. Thirty ml 3:1 chloroform:methanol were used to rinse the silicic acid column sides. Nitrogen gas (5 psi) was applied to pack the column, and liquid was drained to within 5 cm of the top of the silicic acid. Sodium sulfate (anhydrous) was added to a height of 2.5 cm in the

column, and 300 ml chloroform were used to rinse the column. The lipid sample was added and allowed to flow into the packed silicic acid column. The column was developed with 300 ml portions of 1) 20:1, chloroform:methanol, 2) 1:1 chloroform:methanol, and 3) methanol. The solvents were allowed to flow at a rate of approximately 3-5 ml/min. Two fractions were analyzed for fatty acid composition: the 20:1 portion containing neutral lipids, and the combined 1:1 portion and methanol fractions containing the polar lipids.

Fatty Acid Analysis by GLC

Methyl esters of fatty acids of neutral and polar lipids were made by the A.O.C.S. method Ce 2-66 (AOCS 1971) for analysis on a Bendix Gas Liquid Chromatograph Model 2600 equipped with a flame ionization detector and Dohrman recorder with a built-in disc integrator. Fractions from the silicic acid column were evaporated to dryness on the rotary evaporator and made up to volume (25 ml) with 20:3 $CHCL_3$:MEOH for neutral lipid fractions and 1:1 $CHCL_3$:MEOH for polar lipid fractions. A portion containing approximately 100 mg lipid was placed in a 125 ml ground glass stoppered Erlenmeyer flask and evaporated on the rotary evaporator. Four ml 0.5 N NaOH in methanol were added to the flask and refluxed for 10 min. Five ml of boron triflouride in methanol were added through the condenser tube and refluxed for five more min. Five ml of heptane were then added through the condenser, and the mixture heated 1 min and disconnected. Sufficient saturated NaCl solution was added to float the

ester in the neck of the flask. The heptane solution of methyl esters was separated and concentrated under a stream of nitrogen to less than 1 ml. Sodium sulfate was added and the tube flushed with nitrogen and stored until analyzed. (Fatty acid esters were analyzed within 24 hr after preparation.)

A 1.83m x 4 mm inside diameter stainless steel column was packed with 15% DEGS X (diethyline glycol succinate) on a 60/80 mesh acid washed chromasorb W obtained from Applied Science Laboratory, State College, PA. Conditions of the GLC were:

Nitrogen flow rate	40 ml/min
Hydrogen flow rate	40-50 ml/min
Air flow rate	400-500 ml/min
Injection temperature	245°C
Detector temperature	240°C
Initial temperature	neutral lipids-165°C polar lipids-120°C
Final temperature	neutral lipids-195°C polar lipids-220°C
Rate	4°C/min

Approximately 4-6 µl of ester solution were injected into the column. Relative percentages of fatty acids were determined in duplicate. Standards of methyl esters of fatty acids were obtained from Applied Science Lab., State College, PA. They included methyl esters of C_{14}, $C_{14:1}$, C_{16}, $C_{16:1}$, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:2}$, and others not identifiable in the lipid esters. The retention times of the methyl ester standards were used to identify the fatty acids present in the ground beef lipid fractions.

II. SENSORY EVALUATION

Six taste panel members were selected from thirty potential candidates on the basis of four screening tests that were conducted during August, 1976. The screening test was used to check: 1) the panelist opinion of frozen ground beef patties; 2) panelist ability to distinguish repeatedly between grass-fed and grain-fed ground beef; 3) the availability of the panelist for 26 taste panels, 13 in September 1976, and 13 in February 1977.

A score sheet was designed based on the premise that information desired from taste panels in this study was 1) whether there was a flavor difference within each trio, 2) the magnitude of that difference, and 3) whether or not that difference changed over storage time. At each taste panel session, panelists were presented three samples which represented the three animals in a single trio. The score sheet utilized a seven point scale in which panelists were asked to compare each sample separately to the reference. Panelists were asked to indicate whether they like the flavor extremely, moderately, or slightly more than, the same as, or slightly, moderately, or extremely less than the references (See Appendix A). The panel scored each of 13 trios at 30 and 180 days frozen storage.

Samples were prepared from thawed ground beef which was shaped into 130 g patties and cooked for 3 min in a Presto Hamburger cooker. A portion of the patties from each of the three samples were served warm to the panel members under red lights. Salt was available for use and water was provided for rinsing between samples.

III. ANALYSIS OF DATA

The data collected in this study was divided into four parts for analysis using the IBM 360/65 facilities at the University of Tennessee. Data I consisted of characteristics not studied over storage times: muscle nitrogen, percent fat, moisture, weight of semimembranosis muscle at point of fabrication, and color values of the brisket fat by Munsell Color Tabs and Hunter L, a and b values of the inside and outside of the 9 cm fat square, plus carotenoid values of brisket fat. In Data I the effects of trio and ration and their interaction were analyzed as shown in Table 1. The effects of Trio and Ration were tested for significance by the Trio x Ration interaction.

Data II consisted of the taste panel scores. Data III was comprised of chemical components studied for all animals over two storage times. These components were expressed on a dry matter basis: percent free fatty acid, carbohydrate content, free amino nitrogen (ninhydrin reactive material), and TBA numbers. Data from one limited fed steer which was characterized as a dark cutter were eliminated because the ground beef was not representative of normal beef.[1] Means were adjusted for unequal classes. The addition of a storage element altered the Analysis of Variance for Data II and III. This data was analyzed according to Table 2. Trio and Ration effects were tested

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[1] Lusby (1977) reported glycogen content of this steer to be 2.19 mg/100 g meat and our analysis for simple carbohydrate at one month storage was 3 µg glucose / L. water extract.

TABLE I

ANALYSIS OF VARIANCE TABLE FOR DATA I

Source	Degrees freedom
Trio	12
Ration	2
Trio x Ration	24
Total	38

TABLE 2

ANALYSIS OF VARIANCE TABLE FOR DATA II AND III

Source	Degrees freedom
Trio	12
Ration	2
Trio x Ration	24
Storage	1
Ration x Storage	2
Trio x Storage	12
Trio x Ration x Storage	24
Total	77

for significance by the Trio x Ration interaction; storage effects by the Trio x Storage interaction; and Ration x Storage effects by the Trio x Ration x Storage interaction.

The fatty acid composition data of six Angus trios (18 animals) was the subject of Data IV. The effects of trio, ration and storage time on fatty acid composition were analyzed according to Table 3. Known fatty acids which were analyzed statistically were myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic. Unknowns were grouped together by retention times. The effects of sources were tested like Data II and III.

When a source of variation was significant through regression analysis, orthogonal comparisons were used to find significant differences between means. Tables 4 and 5 show the comparisons: C1 and Cs1 analyzed means for significance due to type of ration (with the same energy level); C2 and Cs2 compared low energy rations to high energy ration; Cs3 analyzed across treatments for significance due to frozen storage; Cs4 compared the interaction over storage time due to type ration; and Cs5 studied the interaction over storage time due to level of energy.

TABLE 3

ANALYSIS OF VARIANCE TABLE FOR DATA IV

Source	Degrees of freedom
Trio	5
Ration	2
Trio x Ration	10
Storage	1
Ration x Storage	2
Trio x Storage	5
Trio x Ration x Storage	10
Total	35

TABLE 4

ORTHOGONAL COMPARISONS FOR DATA I

Comparisons	Treatments 1	2	3
C1	+1	-1	0
C2	+1	+1	-2

TABLE 5

ORTHOGONAL COMPARISONS FOR DATA II, III, AND IV

Storage	Tmt 1		Tmt 2		Tmt 3	
	1	2	1	2	1	2
Comparisons						
Cs^1	+1	+1	-1	-1	0	0
Cs^2	-1	-1	-1	-1	+2	+2
Cs^3	+1	-1	+1	-1	+1	-1
Cs^4	+1	-1	-1	+1	+2	-2
Cs^5	-1	+1	-1	+1	+2	-2

CHAPTER IV

RESULTS AND DISCUSSION

I. MUSCLE, PROXIMATE ANALYSIS AND FAT CHARACTERISTICS

Mean squares for fat characteristics are shown in Table 6. There was no significant difference in the amount of carotenoid pigments in the fat. Malphrus (1961) reported a higher amount of carotene in carcass fat of pasture-fed steers. The carotenoid pigment mean (Table 7) is largest for Treatment 1, the grass-fed, but the large error term (Table 6) due to a wide range of values in each treatment negated the possibility of a significant difference. However, significant color differences were detected. Using the Munsell color tabs as a reference of white to yellow range, fat from pasture fed steers showed a significantly higher ($P<0.01$) amount of yellow coloring due to type of ration (C1, Table 6). Treatment 1 had a mean of 3.62 for Munsell color score and Treatment 2 had 2.71 (Table 7). These data support Malphrus' (1961) data that fat of pasture fed steers is more yellow than drylot steers. There was also a difference due to level of energy ($P<0.05$).

Hunter color L, a, and b values further show a significant difference in fat color due to type of ration. Mean squares for "b" values of color on both sides of the fat sample were significantly different due to feeding ration, grass versus grain (C1, Table 6). The mean b values (outside fat) for grass-fed steers was 16.73 compared

TABLE 6

MEAN SQUARES FOR FAT CHARACTERISTICS

Source	Degrees of Freedom	Carotenoid	Color Outside Fat			Color Inside Fat			Munsell
			L	a	b	L	a	b	
Treatment	2	102867.5	3236.62	128.62	2937.72	2642.39	126.34	360.08	418.49
C1	1	204506.7	369.38	12.46	5583.13****	.62	252.35	3058.62***	544.65**
C2	1	3229.5	6103.85**	244.15	292.32	5284.15**	.32	1661.54*	292.32*
Error	24	1138181.	1295.25	421.06	199.14	818.22	200.36	327.94	52.88

****P<0.0001

***P<0.001

**P<0.01

*P<0.05

TABLE 7

TREATMENT MEANS OF SELECTED GROUND BEEF AND BRISKET FAT CHARACTERISTICS

	Treatments		
	1	2	3
Muscle weight (#)	12.23	13.08	13.70
Ground beef			
% Protein nitrogen	18.07	17.64	17.77
Moisture (%)	62.44	63.02	61.74
Fat (%)	18.44	18.34	19.49
Brisket Fat			
Munsell Color tabs[1]	3.62	2.71	2.58
Outside Hunter color[2]	L 66.45	65.69	63.42
	a 4.47	4.61	5.07
	b 16.73	13.80	14.68
Inside Hunter color	L 68.21	68.18	65.72
	a 3.65	3.02	3.32
	b 16.68	14.51	14.21
Carotenoid (ug carotenoid/kg fat)	711	534	603

[1] 7 point scale 1-white, 7-yellow;

[2] L = lightness, a = redness +, gray 0, green -, b = yellow +, gray 0, blueness -.

to 13.80 for limited grain-fed (Table 7). The mean b values of the inside fat were 16.68 for grass-fed and 14.51 for limit-fed. Positive Hunter color "b" values indicate yellow in the sample, the greater the value the more yellow the color. "L" values which signify lightness of the fat color differed due to level of energy (Table 6, C2). Fat from full grain-fed steers had lower "L" values ($P<0.05$). No significant differences were found for "a" values.

Table 8 indicates the mean squares of the muscle weight and proximate analyses characterized in this study. Treatment means are found in Table 7. In a separate research project on the animals used in this study, Lusby (1977) noted that steers from the pasture treatment were lighter at slaughter ($P<0.01$) and carcass weights of steers fed an unlimited amount of grain were heavier ($P<0.0001$). The weight of the semimembranosis muscle, intact, was lower ($P<0.05$) for low energy fed animals (Treatments 1 and 2) than the high energy fed animals (Treatment 3). This was the only difference found in the muscle and proximate characteristics. It should be realized that in formulating the ground beef for this project a 20% fat level was desired to eliminate differences due to the finish or fatness of the steers. Mean fat content of the ground beef ranged from 18.34 to 19.49% (Table 7).

II. STORAGE CHARACTERISTICS

The mean squares of the analysis of storage characteristics for differences due to ration, energy level, storage and the interactions are found in Table 9. Means of these characteristics (water soluble

TABLE 8

MEAN SQUARES FOR MUSCLE WEIGHT AND SELECTED GROUND BEEF CHARACTERISTICS

Source	Degrees of freedom	Muscle wt.	Ground Beef		
			Protein(N2)	%Fat	%Moisture
Treatment	2	707.72	6322.34	52402.39	559.56
C1	1	473.88	12018.5	670.15	92.35
C2	1	941.55*	626.16	104134.62	1026.78
Error	24	183.69	5633.25	41320.77	336.95

* $P<0.05$

TABLE 9

MEAN SQUARES FOR CHEMICAL CHARACTERISTICS DURING STORAGE

Source	degrees of freedom	Free Sugars	Ninhydrin	FFA	TBA
Treatment	2	28.83	61.81	56.62	6.42
Storage	1	1425.76	168325.47	188.83	4.78
Treatment x Storage	2	1.93	162.42	37.14	.30
Cs1	1	55.43**	5.88	.27	2.90
Cs2	1	2.23	117.74	112.96*	9.93**
Cs3	1	1425.76****	168325.47****	188.83*	4.78*
Cs4	1	3.01	248.03	47.41	.29
Cs5	1	.84	76.81	26.87	.31
Error Cs1 & Cs2	23	27.15	290.03	27.48	.83
Error Cs3	12	3.86	182.39	24.43	.53
Error Cs4 & Cs5	23	2.79	218.13	17.68	.16

**** $P<0.0001$
** $P<0.01$
* $P<0.05$

carbohydrates, water soluble ninhydrin reactive material, free fatty acid content and TBA numbers) are found in Table 10.

III. WATER SOLUBLE COMPONENTS

The amount of water soluble carbohydrates (free sugars) was higher in limited grain-fed beef samples than grass-fed ($P<0.01$) (Tables 9 and 10). No reported studies were found in which this finding has been observed before. After 180 days freezer storage, the amount of free sugars in the water extract of all treatments more than doubled (Table 10). This was a significant increase ($P<0.0001$; Table 9, Cs3).

There was not a significant difference in the ninhydrin reactive materials (free amino groups) due to ration or level of energy (Table 9). Ninhydrin values increased significantly ($P<0.0001$) during storage (Tables 9 and 10). Studies have indicated that during refrigerator storage free amino acids increase (Locker, 1960; Saffle et al., 1961; Gardner and Stewart, 1966). However, the values of other studies were not analyzed in the same manner and expressed in the same terms as this study (mg glycine equivalents / g meat).

IV. FREE FATTY ACIDS

Free fatty acid content was slightly higher ($P<0.05$) in the low energy ration beef. There was a significant increase ($P<0.05$) from 30 days to 180 days freezer storage in all treatments. An increase

TABLE 10

TREATMENT MEANS FOR CHEMICAL CHARACTERISTICS DURING STORAGE[a]

	Treatments					
	1		2		3	
	S1[b]	S2[c]	S1	S2	S1	S2
Free sugars (ug glucose eq) / g meat	64.06	147.36	80.98	174.61	77.89	161.73
Ninhydrin Reactive Material (mg glycine eq) / g meat	91.98	180.37	88.83	185.66	90.59	187.62
Free Fatty Acid (% oleic)	1.09	1.30	0.89	1.46	0.86	1.01
TBA Number (mg malonaldehyde) / kg meat	0.58	0.84	0.89	1.42	1.36	2.05

[a]Expressed on a dry matter basis

[b]S1 = 30 days frozen storage

[c]S2 = 180 days frozen storage

in free fatty acid content is due to hydrolysis of lipids. The values found in this study fall into the ranges of reported values of Lea (1931), Broumand et al. (1958) and Pearson (1968). After 180 days, the amounts of free fatty acids had not reached the limit which is unacceptable (1.8% oleic acid) as recommended by Pearson (1968).

V. TBA NUMBERS

Thiobarbituric Acid (TBA) numbers were significantly lower for ground beef from cattle fed low energy rations than the numbers of the high energy treatment (Tables 9 and 10). An increase in TBA numbers indicates oxidative rancidity is occuring. During storage TBA numbers increased for all treatments ($P<0.05$), suggesting that deteriorative activity proceeded during freezer storage.

VI. FATTY ACID COMPOSITION

Two fractions of lipids were studied: neutral lipids making up 95% of the total and polar lipids-5%. In the neutral fraction there were significant differences (Table 11) in the relative percents of myristoleic $C_{14:1}$ and palmitoleic $C_{16:1}$ acids ($P<0.05$), stearic acid $C_{18:0}$ ($P<0.01$) and linoleic C18:2 and linolenic $C_{18:3}$ acids ($P<0.001$) due to level of energy (Table 11 and 12).

Grass-fed steers (treatment 1) had higher levels of stearic ($P<0.05$) and linolenic ($P<0.01$) acids in the neutral lipids (Table 13), higher percentages of linolenic ($P<0.0001$) acid in the polar lipids and lower amounts palmitic acid ($P<0.01$) in both lipid fractions than limited-fed steers. Steers on the high energy grain ration had

TABLE 11

MEAN SQUARES FOR FATTY ACIDS OF NEUTRAL LIPIDS

Source	Degrees of Freedom	Mean Squares							
		C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C8:3
Treatment	2	.16	1.28	60.99	4.57	61.62	27.42	2.69	4.04
Storage	1	.002	.03	.88	3.42	6.81	1.51	2.52	.47
Storage	2	.25	.11	10.71	.80	.30	25.92	.10	.01
Cs1	1	.29	1.60	15.82**	3.25	7.85*	.08	.003	16.36**
Cs2	1	.98	23.06****	14.54**	5.22*	15.72**	2.74	22.51***	26.25***
Cs3	1	.03	.41	.96	4.03	3.90	.23	8.12*	8.32*
Cs4	1	.04	1.12	3.19	.95	.11	.68	1.44	.11
Cs5	1	10.65**	2.71	23.32****	1.31	.35	2.98	1.00	.10
Error for Cs1 and Cs2	10	.26	.10	4.02	1.08	5.23	19.42	.15	.19
Error for Cs3	5	.06	.07	.92	.85	1.75	6.57	.31	.06
Error for Cs4 and Cs5	10	.05	.07	.66	.70	1.32	14.16	.08	.12

****P<.0001

***P<.001

**P<.01

*P<.05

TABLE 12

MEAN SQUARES FOR FATTY ACIDS OF POLAR LIPIDS

Source	Degrees Freedom	Mean Squares C:14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Unknowns
Treatment	2	.48	40.49	2.92	14.56	53.58	164.23	15.05	70.69
Storage	1	.14	26.57	3.14	.16	135.26	5.61	.13	145.04
Treatment	2	.37	8.68	.11	1.60	18.25	6.38	.32	25.18
Cs1	1	.69	76.68****	.48	8.73	50.72	14.58	18.17****	121.95
Cs1	1	.26	4.29	5.36	20.37*	56.44	313.88****	11.92****	19.43
Cs3	1	.14	26.57	3.14**	.16	135.16	5.61	.12	145.04***
Cs4	1	.001	.31	.01	2.91	18.22	2.14	.63	3.29
Cs5	1	.75**	17.65	.21	.29	18.27	10.62	.00	47.08
Error for Cs1 and Cs2	10	.40	7.61	1.27	2.34	40.28	10.15	3.48	69.54
Error for Cs3	5	.23	8.16	.26	1.38	33.99	16.03	1.72	5.72
Error for Cs4 and Cs5	10	.12	6.40	.28	4.92	27.84	9.14	1.18	25.19

****P<.0001

***P<.001

**P<.01

*P<.05

TABLE 13

FATTY ACID COMPOSITION OF NEUTRAL LIPIDS

Fatty acids	Treatments 1 S1[a]	1 S2[b]	2 S1	2 S2	3 S1	3 S2
	relative percents					
$C_{14:0}$	3.36	3.20	3.46	3.23	2.99	3.33
$C_{14:1}$	2.05	1.78	1.76	1.74	1.23	1.34
$C_{16:0}$	24.20	24.07	28.05	26.74	27.28	29.66
$C_{16:1}$	6.87	5.69	7.30	6.79	5.90	5.74
$C_{18:0}$	14.06	13.19	11.60	10.42	9.39	8.83
$C_{18:1}$	46.24	46.10	45.49	47.87	50.74	47.27
$C_{18:2}$	1.24	1.62	1.15	1.75	1.77	2.43
$C_{18:3}$	1.93	2.09	1.17	1.42	.72	1.00

[a]S1 30 days frozen storage

[b]S2 180 days frozen storage

higher levels of palmitic ($P<0.01$) and linoleic ($P<0.001$) acids and lower levels of myristoleic ($P<0.0001$), palmitoleic ($P<0.05$, stearic ($P<0.01$) and linolenic ($P<0.01$) acids in neutral lipids than steers fed the low energy rations (Table 13). Steers on high energy ration also had a higher level of linoleic acid ($P<0.0001$) and lower levels of stearic ($P<0.05$) and linolenic ($P<0.0001$) acids in polar lipids than steers fed low energy rations (Table 14).

Tables 15 and 16 show the grouping of fatty acids according to saturation. Neutral lipids of the grass-fed treatment had a greater percentage of polyunsaturates ($P<0.01$) than the limited fed treatment. Polar lipids of the grass-fed samples had a higher percentage of polyunsaturates ($P<0.05$) than those of the limited grain lipid samples of Treatment 2. However, full grain samples had the highest amount of polyunsaturates in polar lipids of the three treatments and Cs2 (Table 15) shows the level of significance to be $P<0.01$.

VII. CHANGES IN FATTY ACID COMPOSITION DURING FROZEN STORAGE

During frozen storage deteriorative reactions occur in lipids. Already acknowledged in this study have been increases in the amount of free fatty acids indicative of lipid hydrolysis, and increases in the TBA numbers suggesting oxidative rancidity occurred. A decrease in the percent of one or more fatty acids thus may occur. When expressing the amounts in relative percents, a decrease is recognized as a true decrease, but this decrease is off-set by an increase in

TABLE 14

FATTY ACID COMPOSITION OF POLAR LIPIDS

Fatty acids	Treatments 1 S1[a]	1 S2[b]	2 S1	2 S2	3 S1	3 S2
	relative percents					
$C_{14:0}$	1.73	1.41	2.08	1.74	1.42	1.70
$C_{16:0}$	21.25	18.79	25.06	22.14	24.43	22.66
$C_{16:1}$	2.58	1.84	2.82	2.16	1.72	1.34
$C_{18:0}$	13.62	12.92	11.72	12.41	11.27	10.88
$C_{18:1}$	36.62	29.70	37.49	34.35	32.74	30.88
$C_{18:2}$	9.06	11.22	8.10	9.06	15.99	15.25
$C_{18:3}$	3.02	2.57	.95	1.15	.75	.65
Unknowns	12.12	22.57	11.78	18.00	11.69	16.65

[a]S1 30 days frozen storage

[b]S2 180 days frozen storage

TABLE 15

MEAN SQUARES FOR FATTY ACIDS GROUPED BY LEVEL OF SATURATION

Source	Degrees of Freedom	Neutral: Saturates	Neutral: Monounsaturates	Neutral: Polyunsaturates	Polar: Saturates	Polar: Monounsaturates	Polar: Polyunsaturates	Polar: Unknowns
Treatment	2	3.53	9.37	1.67	26.35	78.86	134.38	70.69
Storage	2	2.65	10.56	5.18	35.22	179.60	4.04	145.04
Treatment*Storage	2	18.24	22.32	0.18	10.56	20.75	5.43	25.18
Cs1	1	3.37	7.33	3.18**	43.98	61.12	65.31*	121.95
Cs2	1	3.69	11.41	.15	8.73	96.61	203.45**	19.43
Cs3	1	2.65	10.56	5.18**	35.22*	179.60*	4.04	145.04*
Cs4	1	3.18	17.78	.21	1.25	19.08	.45	3.29
Cs5	1	33.32*	26.86	.15	19.88	22.42	10.42	41.08
Error Cs1 and Cs2	10	7.17	21.70	..28	14.19	49.13	10.08	69.54
Error Cs3	5	.88	7.12	.31	4.59	36.78	16.64	5.72
Error Cs4 and Cs5	10	2.09	8.92	.23	5.39	30.82	7.84	25.19

**P<.01

*P<.05

TABLE 16

FATTY ACIDS GROUPED BY LEVEL OF SATURATION OF POLAR AND NEUTRAL LIPID FRACTIONS

	Treatments					
	1		2		3	
	S1[a]	S2[b]	S1	S2	S1	S2
	relative percents					
Neutral:						
Saturates	41.63	40.65	43.10	40.47	39.65	41.92
Monounsaturates	55.16	56.48	54.54	56.86	57.85	54.34
Polyunsaturates	3.21	3.87	2.36	3.67	2.50	3.73
Polar:						
Saturates	36.60	33.12	38.86	36.28	37.12	35.23
Monounsaturates	39.20	31.54	40.31	36.51	34.46	32.22
Polyunsaturates	12.08	13.78	9.05	10.21	16.75	15.90
Unknowns	12.22	21.56	11.78	17.00	11.67	16.15

[a]S1 = 30 days frozen storage

[b]S2 = 180 days frozen storage

one or more of the remaining fatty acids. Increases are not likely to occur. No results were found in th literature that documented decreases or changes in fatty acids of lipids during freezer storage.

Differences due to storage were observed in the neutral lipid fraction for linoleic and linolenic acids ($P<0.05$, Tables 11 and 13). However, these differences were increases over storage time. In the polar fraction there were significant decreases for palmitoleic acid ($P<0.05$, Tables 12 and 14). A decrease in oleic acid approached significance. The amount of unidentifiable fatty acids of polar lipids increased significantly ($P<0.0001$) during storage time for all three treatments. The amount of polyunsaturates increased in neutral lipid fractions of the three feeding treatments ($P<0.01$, Tables 15 and 16). In the polar fractions saturates and monounsaturates decreased significantly ($P<0.05$).

Several interactions of the storage effects on samples of the treatments were significant. In the neutral fraction the amount of $C_{14:0}$ of low energy ration samples decreased while the amount in high energy grain samples increased ($P<0.01$, Tables 11 and 12). Palmitic acid in the neutral lipid fraction decreased over storage time for the low energy treatments while the relative percentage increased for the high energy treatment. In the polar lipid fraction there was a decrease in the amount of myristic acid in the grass-fed (low energy) ration and the limited-grain (low energy) ration, but an increase in the full-

grain (high energy) ration. This interaction was significant ($P<0.05$, Tables 13 and 14).

An examination of the analysis of the fatty acid composition as grouped by degree of saturation reveals significant ($P<0.05$) storage x energy level interactions for the saturates of the neutral fraction (C_{s5} of Table 15). Relative percents of saturates decreased for low energy treatments and increased in the high energy (Tables 15 and 16).

VIII. TASTE PANEL RESULTS

Analysis of taste panel scores is found in Table 17. There was a significant difference ($P<0.001$) in the flavor of meat due to type of ration and to the level of energy. Taste panel scores did not vary appreciably over frozen storage. The means (Table 18) at 30 days storage show pasture fed beef had a lower score followed by full fed, then limited fed treatments. After 180 days, pasture fed was still lower, yet limited fed beef was now rated second followed by full fed grain beef samples. This shift in preference over storage was not significant.

The panel was selected on the basis of their ability to repeatably distinguish between grass-fed beef and grain-fed beef. Panel scores indicate that grass-fed beef was less desirable consistently. Although deteriorative activity proceeded during frozen storage (according to the values for the chemical characteristics during storage), the deterioration was not perceptable to the taste panel. It would be worthy to

TABLE 17

ANALYSIS OF VARIANCE OF TASTE PANEL SCORES[1]

Source	Degrees freedom	Mean square
Treatment	2	142.028
Storage	1	.320
Treatment x storage	2	3.455
Cs1	1	133.38***
Cs2	1	47.98***
Cs3	1	.30
Cs4	1	1.95
Cs5	1	3.56
Error for Cs1 & Cs2	23	1.538
Error for Cs3	12	8.388
Error for Cs4 & Cs5	23	1.174

[1]6 member screened panel

*** $P<0.001$

TABLE 18

TREATMENT MEANS OF TASTE PANEL SCORES[1] FOR TWO STORAGE TIMES

Treatment	30 day storage	180 day storage
Pasture	2.81	3.00
Limited fed	4.58	4.43
Full fed	4.38	4.81

[1]7 point scale: 1 = extremely better than reference; 4 = same as reference; 7 = extremely less than reference.

note that panel members often complained of an off-flavor in one sample (identified as the grass-fed treatment sample), described as fishy or milky, which left an after taste. This sometimes masked their ability to evaluate other samples separately. Comments from one or two panel members indicated there was only a small difference in the three samples when the grass fed beef sample was the reference of the trio presented. When the reference was a grain-fed sample, the difference in samples was greater.

CHAPTER V

SUMMARY

Thirty-nine steers were divided into thirteen trios according to breed, body type and weight. These were all wintered on a silage ration and in the spring, one member of each trio was assigned to a dry lot and allowed to consume an unlimited grain (high-energy) ration, another was placed on a pasture of orchard grass and fescue for unlimited grazing (low energy grass ration), and the third was allowed a limited amount of grain (low energy ration) to gain at the same rate as the pasture fed group.

Color of carcass fat was significantly more yellow for pasture fed beef than grain fed as judged by two different objective color measurements, both by Munsell color tabs and Hunter Color "b" values. However, there was no significant difference in the amount of carotenoid pigments in the fat. Muscle weights of the semimembranosis were lower for the low energy rations (limited grain and pasture).

Flavor and selected chemical characteristics of ground beef prepared from the semimembranosis muscle and fat trim of the brisket from the left side of each carcass were determined at 30 and 180 days storage at -18°C. Free sugar content was higher in grain fed beef samples than pasture fed. TBA numbers were lower for the low energy rations. There was a slightly higher amount of free fatty acids in ground beef of low energy fed cattle. There was a significant increase in free sugars, free amino groups, free fatty acid content and TBA

numbers over 180 days freezer storage.

Fatty acid analysis was performed on 18 Angus ground beef samples (representing six trios). Pasture fed steers had more polyunsaturated fatty acids in neutral and polar lipids when compared to limited fed steers. Full grain samples had the highest amount of polyunsaturates in the neutral fraction. Decreases occurred in palmitoleic acid in the polar fraction during frozen storage. Relative percents of saturates decreased for low energy treatments.

A selected panel of six members consistently scored ground beef from steers finished on pasture approximately 1.3 to 1.4 units lower on a 7 point scale (1 = dislike extremely more than the reference to 7 = like extremely more than reference) than ground beef from steers finished on a limited or full grain ration. No differences in flavor scores were found for storage.

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APPENDIX

GROUND BEEF

You have a REFERENCE and two separate ground beef samples. First, rinse your mouth. Smell and Taste the reference. Rinse your mouth again. Smell and taste one of the other samples. Under the sample number check the point which best describes your opinion of the flavor as compared to the reference. Rinse your mouth, then smell and taste the reference and compare the remaining sample in the same manner.

Sample #	_____	_____
Extremely better than reference	_____	_____
Moderately better than reference	_____	_____
Slightly better than reference	_____	_____
The same as reference	_____	_____
Slightly less than reference	_____	_____
Moderately less than reference	_____	_____
Extremely less than reference	_____	_____

Feel free to make any comments that you might have concerning the reference or the other samples : _____

_____

_____

Thank you for your cooperation.
See you again _____
for another taste panel.

VITA

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