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Flavor and chemical characteristics of frozen ground beef from steers finished on forage and grain rations

Mohammad Amiri

To the Graduate Council:

I am submitting herewith a dissertation written by Mohammad Amiri entitled "Flavor and chemical characteristics of frozen ground beef from steers finished on forage and grain rations." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

J.T. Miles, Major Professor

We have read this dissertation and recommend its acceptance:

S.L. Melton, H.O. Jaynes, C.C. Melton, W.R. Backus

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B

FLAVOR AND CHEMICAL CHARACTERISTICS OF FROZEN GROUND BEEF FROM STEERS FINISHED ON FORAGE AND GRAIN RATIONS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Mohammad Amiri

March 1980

DEDICATION

With love and appreciation for their ever-present praise, guidance and support, I joyfully dedicate this dissertation to my parents, Dr. and Mrs. Hosein Amiri, whose interest and constant attitude of concern will always be cherished.

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ABSTRACT

Ninety-five steers were grouped into 19 quintets on the basis of breed, weight and body type. One steer of each quintet was finished on a silage-limited grain ration (T1) and another of each quintet was finished on a grain ration during the 1978 winter (T2). The other three steers were wintered on pasture and in April 1978, one of each quintet was finished either on orchard grass, fescue and clover pasture (T3), a limited grain ration (T4) or a full grain ration (T5) during the 1978 summer.

Ground beef containing approximately 20 percent fat was prepared from the semimembranosus muscle and brisket fat of the left side of each carcass. Simple carbohydrates, free amino compounds, fatty acid composition of neutral and polar lipids, flavor score, moisture and fat content were determined for ground beef prepared from steers in T1 through T5. Bacterial count was determined for ground beef prepared from T3, T4 and T5. Changes in simple sugar content and free amino compounds were studied as a function of frozen storage time on T3, T4 and T5. Flavor profile, volatile analysis of heated lyophilized water extract and Hunter color for raw and cooked patties were studied for ground beef prepared from 18 Hereford steers, 6 each for T3, T4 and T5.

No significant difference was found for moisture or fat content among treatments. Ground beef from the low energy level T3 and T4 had a higher population of psychrophilic and lipolytic bacteria than ground beef from high energy T5. Ground beef from summer, grass-fed steers (T3) had less sugar, lower flavor score and higher linolenic acid in

neutral and polar lipids. Other differences in the fatty acid composition of neutral and polar lipids among treatments were also observed. No significant differences among treatments were found for free amino compounds. There was an increase in free amino compounds and a decrease in simple sugar content during frozen storage time.

A dairy or milky flavor which was observed by the flavor profile panel in grass fed beef was particularly unpleasant at higher intensities. Also, there was a lack of beef fat flavor in grass-fed beef. No significant qualitative or quantitative differences were found between volatiles of heated lyophilized water extract of ground beef prepared from grass-fed steers and that of grain-fed steers.

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CHAPTER I

INTRODUCTION

In the United States future indications are that the use of forage to finish cattle will be maximized in order to produce a more economical beef supply. Also, changes in USDA quality grades and increased consumer awareness of reduced dietary fat intake will encourage shorter grain feeding periods for feedlot animals. However, there have been complaints of off flavor in meat from cattle finished completely on grass (grass-fed), and even though there have been conflicting results concerning the flavor of grass-fed vs grain-fed cattle, numerous researchers have reported that grain-fed cattle produce carcasses with superior flavor when compared with carcasses obtained from cattle produced on high levels of forage.

It is difficult to objectively evaluate beef flavor, and many factors have been shown to affect it. Beef flavor is an artifact produced by heating a heterogenous system of nonodorous precursors. Lean meat flavor precursors are the water soluble diffusate compounds present in meat extracts. Nonenzymatic browning reactions between non-protein nitrogen compounds and water soluble carbohydrates are important contributors to meat flavor but may not be the sole mechanism by which meat flavor is developed. Most of the volatiles identified in studies of cooked beef can be readily accounted for by known reactions of the amino acids and sugars identified in beef water extract. Also, the similarity in composition of the free amino acids and reducing sugars in beef, pork and lamb and the similarity in organoleptic qualities

obtained from water extracts of these meats suggest that a basic meaty flavor is common to the lean portion of all meats regardless of species. Lipid portions of meat have been shown to influence the flavor in several ways. Lipid portions of meat have been shown to contain the flavor components that are associated with the development of flavor of different species.

Oxidation, principally of the unsaturated fatty acids, results in the formation of carbonyl compounds that are present in organoleptically significant amounts. Carbonyl compounds may at one level of concentration produce characteristic and desirable flavors and at another concentration level produce undesirable off-flavors.

Phospholipids play a major role in formation of volatile compounds also. Cephalin produces strong, fishy odors, and the odors of combined lecithin-sphingomyelin are somewhat fishy, superimposed on an aroma suggestive of broiled liver. Unsaturated fatty acids with two or more double bonds make up approximately 50 percent of the phospholipid fraction but only about 10 percent of triglyceride fraction. Also, fats serve as a depot for fat soluble compounds that volatalize on heating and strongly effect flavor. The volatiles generated in cooked beef can be swept from the cooked meat, condensed in cold traps or stripped directly into gas chromatograph-mass spectrometer for their isolation and identification. It should be possible to find a relation between chemical compounds in raw meat and the volatiles of cooked meat with the subjective flavor evaluation.

The experiment reported is an investigation of selected chemical characteristics, changes in some of the chemical characteristics as a function of frozen storage time, isolation and identification of some

flavor volatiles and subjective evaluation of flavor of frozen ground beef from steers on different feeding managements.

CHAPTER II

REVIEW OF LITERATURE

I. MEAT FLAVOR

The flavor of meat is an artifact attributed to a complex mixture of compounds produced by heating a heterogenous system containing non-odorous precursors. It is composed of: volatile compounds with odor properties, nonvolatile compounds with taste and tactile properties, potentiators and synergists (Dwivedi, 1975; Hornstein et al., 1967). The factor, however, that exerts the greatest influence on flavor is odor. Meat flavor research, in common with almost all flavor research, has therefore equated odor with flavor and the flavor volatiles, and their precursors have been the system studied (Hornstein et al., 1967).

Precursors of Meat Flavor

Early studies concerned location of the flavor precursors either in fat or lean (Crocker, 1948; Howe and Barbella, 1937; Jones, 1952; Kramlich and Pearson, 1958). Later on, various authors investigated what fractions in the raw meat were responsible for the volatiles (Batzer et al., 1960; Bender et al., 1958; Hornstein and Crowe, 1960; Koehler and Jacobson, 1967; Mabrouk et al., 1969; Wasserman and Gray, 1965; Wasserman and Spinelli, 1970). They usually prepared a cold, aqueous extract of raw meat and separated the high- and low-molecular weight materials by dialysis. Upon heating the two fractions, the low-molecular diffusate produced meat-like aroma. This fraction contained free amino acids and monosaccharides.

Reactions induced by heating sugars and amino acids are "nonenzymic browning" or "Mailard" reactions. Meat flavors are generated in reactions of this type. Various authors separated the diffusate to reveal its amino acid composition (Bender et al., 1958; Batzer et al., 1960; Wasserman and Gray, 1965; Wasserman and Spinelli, 1970). Surprisingly the data reported vary because the substrate was not standardized. Initially, methionine was the only sulfur containing amino acid identified (Bender et al., 1958); however, later studies revealed the presence of cysteine, cystine, or cysteic acid (Koehler and Jacobson, 1967; Wasserman and Spinelli, 1970). Taurine (2-aminoethanesulfonic acid) was also reported as a component of the diffusate (Macy et al., 1964). The main sugar present was glucose, but ribose, deoxyribose and ribose-5-phosphate, fragments of 5'-nucleotides, were also identified (Dwivedi, 1975; Koehler and Jacobson, 1967; Wasserman and Spinelli, 1970). Table 1 shows the potential flavor precursors isolated from meat extracts (Dwivedi, 1975). While enzymatic browning reactions between amino acids and sugars are important contributors to meat flavor, they are not the sole mechanism by which meat flavor is developed. Inter- and intra-molecular cyclization as well as numerous reactions which are made possible by the activity of ammonia, hydrogen sulfide, mercaptans, and other unidentified intermediates, especially at elevated temperatures are also important (Wilson et al., 1973).

Macy et al. (1964) and Wasserman and Spinelli (1972) reported no great differences between the amino acid patterns of the diffusates of beef, pork, and lamb. The meat aromas released when diffusates of these species heated were very similar, provided the fat had been removed before extraction in cold water. This finding stresses

TABLE 1

POTENTIAL FLAVOR PRECURSORS ISOLATED FROM MEAT EXTRACTS

Alanine	Glucose-6'-phosphate	Ornithine
B-Alanine	Glutamic acid	Peptides
Quarternary amines	Glutamine	Phenylalanine
Ammonia	Glutathione	Phosphoethanolamine
Anserine	Glycerophosphoethanol-amine	Phosphoserine
Arginine	Glycine	Proline
Asparagine	Glycoproteins	Purine-nuceosides
Aspartic acid	Histidine	Purine-nucleotides
Carnitine	Hydroxyproline	Ribose
Carnosine	Hypoxanthine	Ribose-5-phosphate
Citrulline	Inosine-5-monophos-phate & other nucleo-tides	Serine
Creatine	Isoleucine	Taurine
Creatinine	Leucine	Threonine
Cysteine	Lysine	Tyrosine
Cystine	Methionine	Urea
Fructose	Methylhestidine	Valine
Fructose-6-phosphate	Nicotinamide-adeninedinucleatide	
Glucose		

(Dwivedi, 1975)

importance of fat in determining the characteristic species flavor. It is not unexpected that unsaturated aldehydes derived from unsaturated fatty acids in the fat contribute to the typical species flavor. Fat is also capable of dissolving the nonpolar volatile compounds, thus providing a reservoir of flavor compounds (Pippen, et al., 1969). Of course, reactions affecting flavor can take place between the compounds soluble in the fat as well as reactions between the compounds and the fatty acids of the fat (Lien and Nawar, 1974).

Fatty Acid Composition Relationship to Flavor

A number of studies have been conducted which show a relationship between fatty acid composition and meat palatability (Dryden and Marchello, 1970; Waldman et al., 1965; Waldman et al., 1968). Oxidation and hydrolysis of lipids, particularly polar lipids, in meat during refrigerated and frozen storage have been generally responsible for undesirable flavor (Jackobsson and Bengston, 1973; Lea, 1960; Pearson, 1968). Hornstein et al. (1961) determined the lipid composition of lean beef and pork and related their findings to flavor. Westerling and Hedrick (1979) reported high positive correlation coefficients between flavor score and either intramuscular oleic (C18:1) acid or intramuscular total unsaturated fatty acids and high negative correlation coefficients between flavor score and each of the following intramuscular fatty acids: palmitic (C16:0), stearic (C18:0), linoleic (C18:2) and total saturated fatty acids. Brown et al. (1979) proposed that linolenic acid (C18:3) contributed to the low flavor score of grass-fed beef. Also, the possibility exists that microbial growth on beef which has higher linolenic acid could contribute to the inferior

flavor. Pseudomonas fragi, one of the most predominant species found on beef carcasses (Stringer et al., 1969) produces large amounts of alkanals, 2-alkenals and 2-alkones (Smith and Alford, 1969). These compounds are known to contribute off flavors to foods (Forss, 1969; Labuzza, 1971).

II. VOLATILE CONSTITUENTS OF BEEF

Many investigations have been devoted to the isolation and identification of volatile constituents of beef. The volatiles of beef were investigated by Bender and Ballance (1961), Burks et al. (1959), Chang et al. (1968), Hirai et al. (1973), Hornstein et al. (1960), Hornstein and Crow (1960), Kramlich and Pearson (1960); Lieblich et al. (1972), Merrit et al. (1959), Mussinan et al. (1973), Pearson and Von Sydow (1973), Sanderson et al. (1966), Stahl (1957), Tonsbeek et al. (1968), Watanabe and Sato (1971, 1972), and Wilson et al. (1973). The volatiles of meat have been also extensively studied by Dwivedi (1975), Herz and Chang (1970) and Wang and Ordell (1973).

Gas liquid chromatography (GLC) has been the technique used in all volatile analysis and a list of over 200 volatile constituents in the aroma of heated beef have been compiled (Dwivedi, 1975). These include members of at least 17 different chemical classes. Table 2 shows a number of volatile compounds in heated beef (Dwivedi, 1975).

Different investigators have been able to isolate and identify different constituents in beef volatiles. Stahl (1957), in the course of a study on irradiation flavor damage in beef, analyzed unirradiated raw beef volatiles. Vacuum fractionation was used to collect

TABLE 2

NUMBERS OF VOLATILE COMPOUNDS IN HEATED BEEF

Class	
Acids	6
Alcohols	25
Aldehydes	31
Benzene Compounds	12
Esters	3
Ethers	1
Furans	8
Hydrocarbons	19
Ketones	23
Lactones	11
Pyrazines	32
Pyrroles	2
Sulfides	8
Thiazoles	10
Thiols	11
Thiophenes	16

(Dwivedi, 1975)

condensables of increasing volatility in successively colder traps. Fractions were separated by GLC. Hydrogen sulfide, methyl mercaptan, ethyl mercaptan, carbon dioxide, and carbon monoxide were then identified by mass spectrometry. In a similar technique, Merritt et al. (1959) reported hydrogen sulfide, methyl mercaptan, ethyl mercaptan, acetaldehyde, acetone, 2 butanoe, methanol and ethanol in unirradiated raw beef volatiles. Burks et al. (1959) used both paper chromatography and GLC to study the amine volatiles of raw beef. The volatiles consisted of 99.9 percent ammonia and .1 percent of an unidentified compound with a retention time less than that of ammonia. Hornstein et al. (1960) and Hornstein and Crowe (1960) blended lean beef with water at 0°C, filtered the slurry, lyophilized the filtrate, and obtained a hygroscopic powder. This powder was heated to 100°C under 10^{-5} torr. The volatiles were trapped in liquid nitrogen and the total condensate was fractionated under vacuum to yield two major fractions: a highly volatile fraction, collected at -196°C, which was not very desirable and contained ammonia, traces of methyl amine, hydrogen sulfide, methyl mercaptan, acetone, formaldehyde, acetaldehyde, and several unidentified highly volatile compounds and another fraction with a rather desirable aroma which was less volatile and was the remaining residue in the trap in which the total volatiles were initially condensed. Lactic acid and its ammonium salt were the only compounds positively identified in this fraction. Kramlich and Pearson (1960) refluxed a mixture of ground beef and water; generated volatiles were swept from the reaction flask by a stream of nitrogen, condensed in cold traps, and identified by GLC. Methyl mercaptan, methyl sulfide, acetaldehyde, and acetone were identified by retention volumes and

chemical tests. Yueh and Strong (1960) also refluxed a mixture of ground beef and water, filtered the mixture, adjusted the broth pH at 1, and distilled the volatile organic acids. The acids were esterified with diazomethane and methyl esters were separated by GLC on diisodecylphthalate. Formic, acetic, propionic, and 2-methyl propionic acids were identified by comparison of the retention times of unknowns with those of authentic methyl esters. When similar broth was distilled at pH 5 to 6, methyl sulfide, ammonia, acetone, acetaldehyde, diacetyl and hydrogen sulfide were separated. Bender and Ballance (1961) studied the volatiles from a commercial beef extract. Identified volatile compounds included hydrogen sulfide, methyl mercaptan, acetaldehyde, propionaldehyde, 2-methylpropanal, acetone, 3-methylbutanal, 2-butanone, ethyl mercaptan, dimethyl sulfide, methanol and ethanol. Herz and Chang (1970) reported that lactones, furan ring compounds that do not contain sulfur, and aliphatic sulfur compounds made a direct contribution to the meat flavor profile but they conceded that none of the representatives of these classes of compounds has a characteristic meaty aroma. Watanabe and Sato (1971) reported that in shallow fried beef, alkyl-substituted pyrazines and pyridines are the most likely responsible compounds for the typical roasted flavor. In another study of shallow fried beef flavor, Watanabe and Sato (1972) isolated a typical heated beef flavor and observed that no single compound isolated in the fraction had a typical heated beef flavor. They suggested that the heated beef flavor might be a complex sensation resulting from a mixture of methional, 2-acetyl furan, 2-furfuryl methyl ketone, 1-methyl-2-acetylpyrrole, benzothiazole,

and other compounds. Hirai et al. (1973) isolated the volatiles from beef broth and were able to identify 53 different compounds. They included: hydrocarbons, alcohols, esters, ethers, lactones, aldehydes, ketones, acids, sulfides, aromatic compounds, and hetero-cyclic compounds.

It appears that the nature of compounds responsible for the characteristic cooked meat aroma remains unresolved. While most of the compounds present in cooked meat are likely to play a significant role in determining its flavor character, when presented in a mixture, odor compounds give an odor sensation somewhat different from the one predicted on the basis of odor characteristics of the individual components in the mixture. It may, therefore, be futile to look for the "characteristic" compounds responsible for the typical meat flavor (Dwivedi, 1975).

III. FATTY ACID COMPOSITION OF BEEF AS AFFECTED BY DIET

It was believed for many years that ruminant fat depots were relatively stable and not subject to the influence of diet, sex, environment, etc., as are the tissues of many monogastric species. Garton (1960) reported that dietary unsaturated fatty acids are partially or completely hydrogenated by rumen microorganisms and the degree of unsaturation of body fats in ruminants is effected very little by most diets. However, more recent studies have shown differences in fatty acid composition of ruminant tissues caused by differences in diet (Bensadoun and Reid, 1965; Brown et al., 1979; Cabezas et al., 1965; Church, et al., 1967; Clemens et al., 1973; Cook, 1963; Cramer and Marchello, 1962; Edwards et al., 1961; Erwin et al., 1963; Reiser and Reddy, 1956; Roberts and McKirdy, 1964;

Sumida et al., 1972; Tove and Matrone, 1962; Tove and Mochrie, 1963; Varnell et al., 1965; Westerling and Hedrick, 1979).

There is conflicting information on fatty acid composition as affected by diet. Privett et al. (1965) reported that lower percentages of saturated fatty acids (and higher percentages of oleic and linoleic acids) were found in shoulders and rounds of beef cattle fed high energy diets compared to cattle fed a low level maintenance diet. Edward et al. (1961) found that addition of animal fat to steer rations resulted in a highly significant increase in stearic content of rib fat. Cook (1963) found no difference in fatty acid composition of neutral fraction of raw and cooked beef lipids due to feeding management (grass finished vs grain finished). However, in the phospholipid fraction, three unidentified components were increased in concentration with aging time and these were of greater percentages in fat from grass-fed cattle. To study the effect of physical form of diet on composition of fatty acids in beef Cabezas et al. (1965) studied the effect of the ratio of dried citrus meal to corn meal. Data on rib fat composition revealed that a higher ($P < .05$) degree of unsaturations was found for diets containing 72 percent corn. Church et al. (1967) found addition of tallow fat resulted in significant increases in C14:0, C16:1, C17:0, C18:0, and a decrease of C18:1. Sumida et al. (1972) found feeding treatment effected fatty acid composition but did not effect fatty acids in different sample sites in the same manner. Brown et al. (1979) compared grass-finished steers with steers finished on grain at the same energy level and reported that steers finished on fescue, clover and orchard grass has a higher concentration of linolenic (C18:3) in neutral and polar lipids. Westerling and Hedrick (1979)

reported that intramuscular fat from steers and heifers finished on fescue had a higher amount of linoleic (C18:2) and linolenic than those finished for either 56 or 112 days on a grain diet.

Tove (1960) reported that animal fats may contain more than 35 fatty acids but, that 90 percent of the fat depot was comprised of myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acids. It has been established that these fatty acids may vary according to depot site (El-Gharbawi and Dugan, 1965; Hornstein et al., 1967; Keller and Kinsella, 1973; Marchello et al., 1968; Read et al., 1963; O'Keefe et al., 1968; Terrell et al., 1967).

IV. MICROBIAL CONTAMINATION OF BEEF

Bacterial contamination of the beef carcass may be derived from several sources such as the intestinal tract, hide, lymph nodes, localized infections on the animal, and slaughter knife. The vast majority of bacteria present on the carcass surface and in the intestinal tract of animals are removed during slaughter operations. Those remaining on the eviscerated and skinned carcass derive from the animal itself, and to a lesser extent from soil and water (Speck, 1976).

The intestinal tract is the most important source of bacteria. It contributes Clostridium perfingens, coliforms, Salmonella, and Staphylococcus to the meat surface (Grau et al., 1968).

The hide also harbors massive numbers of intestinal, soil and water bacteria. The killing, dressing, and washing operations vastly reduce this microbial load. Yet a few of the bacteria from the outside of the animal unavoidably become part of the surface flora of the carcass. This flora contains mainly harmless mesophiles, low numbers

of food poisoning or animal disease pathogens, and harmless psychrotrophic bacteria (Speck, 1976).

Lymph nodes frequently contain animal pathogens filtered from the lymph fluid (Rubin et al., 1942). They may contain any of the animal disease organisms, such as members of the genera Staphylococcus, Clostridium, Streptococcus, Bordetella, Corynebacterium, Mycobacterium, Salmonella and Pseudomonas, and all of them remain as part of the carcass (Speck, 1976).

Bruising of the live animal, which encourages infection by Staphylococci, and to a lesser extent other bacteria, causes localized infections in animal tissues and also contributes to the carcass bacterial load (May and Handy, 1966).

Also, continued heart action sometimes pulls a few bacteria into the blood stream of red meat animals from the slaughter knife. These bacteria lodge in the deep tissue of the carcass (Haines, 1941).

Psychrophilic Microorganisms

This group of microorganisms grow in foods at refrigeration temperatures. Although the term implies optimum growth at low temperatures, relatively few of psychrophilic microorganisms isolated from foods have optimum growth temperatures below 20°C (Tompkin, 1973).

The term "psychrophilic" is generally applied to those organisms that are able to grow relatively rapidly at commercial refrigeration temperatures without reference to optimum temperature for growth (Mossel and Zwart, 1960). Species of Pseudomonas, Achromobacter, Flavobacterium and Alcaligenes are often included among the psychrophilic bacteria.

The enumeration of psychrophilic bacteria in foods that are to be stored refrigerated (0° to 10°C) is important. Many psychrophilic bacteria when present in large numbers can cause a variety of off flavors as well as physical defects in foods. Their growth rate is highly temperature dependent, and becomes increasingly slower as the temperature is reduced. Therefore, shelf life or the rate of quality loss, and subsequent spoilage of a refrigerated food is also highly temperature dependent (Elliott and Michener, 1965; Tompkin, 1973).

The genera Pseudomonas among bacteria is the most frequently reported psychrophilic microorganism in fresh meats (Jay, 1978).

Lipolytic Microorganisms

The foods that contain fat are susceptible to hydrolysis and oxidation of the fat portion which leads to changes in flavor. Although many of the problems of fat breakdown are nonmicrobial in origin, numerous bacteria, yeasts and molds are capable of causing both hydrolytic and oxidative deterioration.

The genera Pseudomonas, Achromobacter and Staphylococcus among bacteria, Rhizopus, Geotrichum, Aspergillus, and Penicillium among molds, and yeast genera Candida, Rhodotorulla, and Hansenula contain many lipolytic species (Bours and Mossel, 1973; Lawrence, 1967). The genera Pseudomonas among bacteria is the most frequently reported lipolytic microorganism in fresh meats (Jay, 1978).

Lipolytic counts usually are not performed on a routine basis in food manufacturing, but they are essential for flavor studies since flavor changes may occur in the fat of the food.

Proteolytic Microorganisms

Hydrolysis of protein by microorganisms in food may produce a variety of odor and flavor defects. Some of the common psychrotrophic spoilage bacteria are strongly proteolytic and cause undesirable changes in meat particularly when high populations are reached after extended, refrigerated storage. On the other hand, microbial proteolytic activity may be desirable in some foods. There are different opinions concerning the usefulness of proteolytic counts to evaluate quality losses of refrigerated meat products (Jay, 1972; Levin, 1968; Martley et al., 1970).

Proteolytic species are common among the genera Clostridium, Bacillus, Pseudomonas, and Proteus. Microorganisms that carry out protein hydrolysis and acid fermentation are called acid proteolytic, examples of this group are: Streptococcus faecalis variety liquefaciens and Micrococcus caseolyticus (Frazier, 1967). The genera Pseudomonas among bacteria is the most frequently reported proteolytic microorganism in fresh meat (Jay, 1978).

V. MEAT COLOR

There have been reports on differences of carcass color due to type of ration. Malphrus (1961) reported a higher amount of carotene in carcass fat of pasture fed steers. Brown et al. (1979), using the Musell color tabs as a reference of white to yellow range, also reported higher amount of yellow coloring in carcass fat of pasture-fed steers compared to the fat from drylot steers. Lusby (1977) noted that meat from steers on pasture were lighter in color at slaughter compared to meat from steers from drylot.

Meat color has been generally measured to follow visual changes which meat undergoes, or to obtain a measure of the chemical state of myoglobin and its derivatives by means of colorimetric techniques. In cooked meat beside the chemical state of myoglobin and its derivatives, nonenzymatic browning reactions between amino acids and sugars are also important contributors to meat color (Clydesdale and Francis, 1971).

Different techniques have been used to measure the meat color. Kraft and Ayres (1954) used a Beckman Model D spectrophotometer with a diffuse reflectance attachment to obtain readings in the region between 540 and 800 nm which they considered a measure of the change in meat color. Mangel (1951), Butler et al. (1953), and Broumand et al. (1958) used a technique by which the myoglobin derivatives in aqueous extracts of meat were determined spectrophotometrically. Dean and Ball (1960) believed that a method based on the same principles as Broumand's method, but depending upon reflectance instead of absorbance or transmittance, might give more reliable results since the surface of the meat could be measured directly, without requiring an extraction. Snyder (1964) suggested a method for the precise measurement of discoloration in fresh meats which uses "a" values obtained from a Gardner automatic color-difference meter. He found in changing from oxymyoglobin or myoglobin to metmyoglobin, the "a" values decrease. Using the "a" value only, it would be difficult to determine whether the oxymyoglobin in meat sample was oxidized to metmyoglobin or deoxygenated to myoglobin, since both changes represent a decrease in "a" values. However, the two types of change can be distinguished by considering the a/b ratio. For oxymyoglobin and myoglobin, which are both fresh meat colors, the a/b ratio does not change appreciably, but for conversion of oxymyoglobin

or myoglobin to metmyoglobin the a/b ratio decreases considerably. This results because in this conversion there is a considerable change in the "a" values, but relatively little change in the "b" values (Clydesdale and Francis, 1971).

CHAPTER III

MATERIALS AND METHODS

I. SELECTION OF STEERS, FEEDING MANAGEMENT AND SLAUGHTER

Ninety-five steers weighing 193-238 kg were grouped into 19 quintets on the basis of breed, weight and body type similarities predicting rate of maturity. Steers in each quintet were randomly assigned to one of five feeding treatments.

Two steers from each quintet were fed at The University of Tennessee Blount Farm, Knoxville, Tennessee. From November 1977 until Spring 1978 one of the steers from each quintet was assigned to a dry lot and allowed to consume a silage and a limited grain ration (Treatment 1, low energy, silage and limited grain-fed, winter). The other steer from each quintet was assigned to a dry lot and allowed to consume ad libitum, a full grain ration which its composition is given in Appendix A (Treatment 2, high energy, full grain-fed, winter). The steers in Treatment 1 were slaughtered at an estimated backfat thickness of 6 mm over the 12-13th rib as measured with a Branson Model 12 Sono-Ray, and the steers in Treatment 2 were slaughtered at an estimated backfat thickness of 12 mm.

Three steers from each quintet were wintered on pasture and during inclement weather on stock piled fescue hay from November 1977 through April 1978 at The University of Tennessee Plateau Experiment Station, Crossville, Tennessee. At the time grass became plentiful at Crossville, one of the three steers from each quintet was randomly assigned to one of three feeding treatments. Steers in Treatment 3

were allowed unlimited grazing on a pasture of orchard grass, clover and fescue (low energy, grass-fed, summer). Steers in Treatment 5 were assigned to a dry lot and were allowed to consume an unlimited amount of grain ration (high energy, full grain-fed, summer). Steers from Treatments 3 and 4 were slaughtered at an estimated backfat thickness of 6 mm and steers from Treatment 5 were slaughtered at an estimated backfat thickness of 12 mm.

II. POST MORTEM HANDLING AND GROUND BEEF FORMULATION

The steers were slaughtered over a 6-month period from March through August 1978 at the East Tennessee Packing Company, Knoxville, Tennessee. After the slaughter the carcasses were chilled and transferred at 24 hours post mortem to The University of Tennessee Department of Food Technology and Science. The carcasses were aged for 10 days at 1.6°C before wholesale cut fabrication. At the time of wholesale cut fabrication, the Semimembranosus muscle from the left side of each carcass and external fat from left brisket were removed and each ground through 1/2" plate on a Hobart Model 4722 meat grinder. Then, fat content of the lean ground muscle and ground brisket fat were determined by a Modified Babcock fat analysis (Ockerman, 1969). Ground beef formulated to contain approximately 20 percent by Pearson Square (Terrell, 1971) was prepared from the lean and the fat by grinding them together through a 1/8" plate on the Hobart mixer. After grinding, 50 g samples of ground beef from each steer were placed in whirlpack bags and kept at 1°C for 48 hours for microbial count. Other samples of ground beef from each steer were placed in whirlpack bags, flushed with nitrogen and stored at -6°C for chemical analysis at 0 storage

time. Two 125 g patties were sealed in plastic bags and stored at -6°C for color and moisture loss study. The rest of the ground beef from each steer was divided into 500 g portions and wrapped in waxed freezer paper. The packages were stored at -6°C for future flavor studies and chemical analysis at 6 and 12 months of storage.

III. CHEMICAL ANALYSIS

Within 20 days after ground beef preparation, the samples of ground beef from each steer taken for chemical analysis at 0 storage time were allowed to thaw at room temperature. Total lipids and water soluble compounds were extracted from the thawed ground beef. At each storage period of 6 and 12 months a 500 g portion of frozen ground beef from each steer was thawed at 1°C for 24 hours. The samples for chemical analysis were weighed into Whirlpack bags, flushed with nitrogen, sealed and stored at -6°C for no more than one week. At each storage period, water-soluble materials were extracted from the ground beef samples.

Water Extract of Ground Beef

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

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

Determination of Simple Carbohydrates and Related Substances

The 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 percent phenol solution were added to each tube and the sample mixed well. The test tubes were held at room temperature for 10 minutes and placed in a 25°C water bath for 10 minutes. Absorbance was read at 485 nm on a Hitachi Model 100-60 double beam spectrophotometer. The blank was composed of 2 ml deionized water reacted like the deproteinated extract.

A standard curve was established for glucose solutions from 2×10^{-2} to 18×10^{-2} mg/ml on each day of analysis. The linear regression coefficient for each standard curve was above 0.99.

Ninhydrin Reactive Materials

The ninhydrin reactive material content of the water extract was determined by the method of Mickelson (1969).

Water soluble ninhydrin reactive material was calculated in units of mg glycine equivalents. A standard curve was established for glycine solutions from 5×10^{-3} to 50×10^{-3} mg/ml on each day of analysis. The linear regression coefficient for each standard curve was above 0.99.

The following solutions were used:

1. Ninhydrin reagent: 95 g KH_2PO_4, 43 g Na_2PO_4, 5 g triketohydrindene hydrate, and 3 g fructose made up to one liter with deionized water.
2. Ninhydrin diluent: 2 g KIO_3, 400 ml 95 percent ethanol, and 600 ml deionized water.

One ml of the deproteinated extract was diluted to 10 ml, one ml of this dilution was mixed with one ml ninhydrin reagent and held in a boiling water bath for 15 minutes. After cooling, 5 ml ninhydrin diluent were added and absorbance read at 570 nm on the Hitachi Model 100-60 double beam spectrophotometer against a blank composed of one ml deionized water treated like the deproteinated extract dilution.

Lipid Extraction

Ground beef total lipids were extracted by a modified procedure of Ostrander and Dugan (1961). Forty g of ground beef were blended with 130 ml reagent grade methanol for 5 minutes. Sixty-five ml of chloroform were poured down the sides of the blender jar and the mixture reblended for 5 minutes. An additional 65 ml chloroform were added and the mixture was blended for 20 seconds. Next, 1.5 g of zinc acetate dissolved in 65 ml of deionized water was added and the blending was continued an additional 10 more seconds. Under a blanket of nitrogen this mixture was filtered through Watman No. 1 filter paper in a Buchner funnel into a suction flask. The filtrate, filter paper and one half tissue used to wipe the funnel were reblended in the same blender jar for 2 1/2 minutes with 100 ml of chloroform. This mixture was filtered through filter paper in the above Buchner funnel into the same suction flask. The blender jar was rinsed with 75 ml chloroform which also was filtered into the suction flask. All of the filtrate was poured into a 500 ml graduated cylinder and the suction flask rinsed with 25 ml of methanol which was added to the filtrate.

The filtrate was transferred to a 500 ml separatory funnel and placed in a 2°C cooler until a sharp interface was apparent between

methanol-water and chloroform layers. The chloroform layer containing the lipids was collected and the solvent was evaporated from the lipid extract on a rotary evaporator at 35°C and vacuum of 380 torr. The dried extracted lipids were transferred quantitatively with 50 ml chloroform to a 50 ml Erlenmeyer flask. The flask was flushed with nitrogen, stopped, and stored at -6°C until the lipids were fractioned into polar and neutral lipids.

Fractionation of Total Lipids

The beef lipids were separated into polar and neutral lipid fractions by a modified silicic acid slurry method described by Murty et al. (1960).

For each fractionation 50 g silicic acid were activated by heating at 110°C for 20 hours and then cooling for one hour in a desiccator prior to use. Fifteen ml aliquots of the concentrated lipid extracts were diluted to 25 ml with chloroform and transferred quantitatively with 50 ml of chloroform to a 500 ml Erlenmeyer flask. Fifty g silicic acid and 200 ml chloroform: methanol (20:1) were added to the flask. A magnetic stirring bar was added and the slurry was mixed for 10 minutes on a magnetic stirrer. A nitrogen atmosphere was provided by nitrogen delivered through an inverted funnel over the sample flask. The slurry was quantitatively transferred to a funnel with a medium sintered glass filter and filtered by suction. Five 50 ml portions of chloroform were used for washing the silicic acid in the funnel. During each washing the suction was released and the silicic acid and the chloroform were stirred. The filtrate containing the neutral lipids was then transferred quantitatively with chloroform to a

1000 ml round bottom flask and dried down to 25 ml as described previously. In case volume was less than 25 ml, chloroform: methanol (20:1) was used to return to volume.

Fatty Acid Analysis by GLC

Methyl esters of fatty acids of neutral and polar lipids were made by the A.O.C.S. method C_e2-66 (AOCS 1971) for analysis on a Bendix Gas Liquid Chromatograph Model 2600 equipped with a flame ionization detector and a Dohrman recorder with a Chromatopac-E1A integrator. A portion of lipid fraction from silicic acid fractionation containing approximately 250 mg lipid was placed in a 125 ml ground glass stoppered Erlenmeyer flask. Four ml 0.5 N NaOH in methanol and 3 boiling beads were added to the flask and refluxed for 10 minutes. Five ml of boron trifluoride in methanol (125 g per liter of methanol) were added through the condenser tube and refluxed for 5 more minutes. Five ml of heptane were then added through the condenser, and the mixture heated one minute and disconnected. Sufficient saturated NaCl solution was added to float the heptane solution containing esters in the neck of the flask. The heptane solution of methyl esters was separated, concentrated and put into a test tube. A small amount of anhydrous sodium sulfate was added and the tube flushed with nitrogen and stored at -10°C until analyzed within 24 hours.

A 1.83 m X 6.35 mm o.d. stainless steel column was packed with 10 percent EGSS-X on a 100/120 mesh Gas-Chrom Q from Applied Science Laboratory, State College, PA. Conditions of the GLC were:

Nitrogen flow rate ---------- 45 ml/min

Hydrogen flow rate ---------- 30 ml/min

Air flow rate ---------- 90 ml/min

Injection temperature ---------- 225°C

Detector temperature ---------- 225°C

Initial temperature ---------- neutral lipids - 190°C

polar lipids - 180°C

Final temperature ---------- neutral lipids - 190°C

polar lipids - 225°C

Rate ---------- 4°C/min

Approximately 4-6 ml of ester solution were injected into the column. Relative percentages of fatty acids were determined in duplicate. Quantitative standards of methyl esters of fatty acids were obtained from Applied Science Lab., State College, PA. They included methyl esters of C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2, C18:3, C22:0, C20:4. The retention times of the methyl ester standards were used to identify the fatty acids present in the ground beef lipid fractions and to determine correction factor in order to quantitatively determine relative percentage composition. Unknown fatty acids were grouped together by retention times. The relative retention times compared to C16:0 were as follows: C14:0, 0.57; C14:1, 0.76; C15:0, 0.79; C16:0, 1.00; C16:1, 1.23: C17:0, 1.42; C18:0, 1.66; C18:1, 1.94; C18:2, 2.45; C18:3, 2.85; C22:0, 3.13; C20:4, 3.31; X, 3.56; Y, 3.81; Z, 4.16.

IV. MICROBIOLOGICAL ANALYSIS

A 50 g sample of ground beef from each steer in Treatments 3, 4, and 5 was used to determine aerobic, psychrophilic, proteolytic, and lipolytic bacterial plate counts using modified methods from Compendium of Methods for the Microbiological Examinations of Foods (Speck, 1976). The counts were made on ground beef that had been stored at 1°C for 48 hours after preparation. The 50 g sample was homogenized with 500 ml of sterile deionized water. For aerobic counts, dilutions of the homogenate were plated on standard plates and the plates were incubated at 32°C for 48 hours prior to counting. Psychrophilic microorganisms were enumerated by plating the dilutions of the homogenate on standard plate with incubation of plates at 5°C for 10 days. For proteolytic counts, dilutions of the homogenate were plated on standard method caseinate agar and the plates were incubated at 30°C for 48 hours. For lypolitic counts, the dilutions of the homogenate were plated on spirit blue agar with incubation of plates at 10°C for 5 days.

V. SENSORY EVALUATION

Taste Panel

Twenty-five untrained panelists subjectively evaluated the flavor of ground beef from 5 different steers at one time on a 9-point hedonic scale from 1 = extremely undesirable to 9 = extremely desirable (Appendix B). Ground beef from each steer was stored at -6°C for no more than 6 weeks prior to sensory evaluation, the timing of each sensory panel evaluation was controlled by the steer slaughter schedule. When 5 or more steers had been slaughtered and the ground beef had been

prepared, a sensory evaluation panel were run. If more than 5 steers had been slaughtered, the ground beef samples were chosen at random for each panel sitting.

Ground beef from 500 g packages was thawed and shaped into 125 g patties. These patties were cooked for 3 minutes to an internal temperature of 68°C on a Presto Hamburger Cooker Model No. 05-MB2. The patties were divided into 8 parts per patty and kept at 53°C in covered dishes on a warming tray for no more than one hour prior to evaluation. Each panelist was served 1/8 of a warm hamburger patty from 5 different steers under red lights in a sensory evaluation booth at one sitting. Salt was available for use and water was provided for rinsing between samples. All sensory panel evaluations were carried out between 2:00 to 4:00 p.m.

Flavor Profile

Flavor profile was determined according to the procedure of Arthur D. Little which was formalized and officially introduced to the food field in 1949 by Cairncross and Sjostrom (1950). For flavor profile determination, ground beef from 18 Hereford steers (6 from each Treatment 3, 4, and 5) was used. The flavor profile panel at North Carolina State University, Raleigh, North Carolina which had been trained to evaluate meat by an Arthur D. Little panel evaluated the ground beef.

Intensity of character notes of aroma, flavor, and aftertaste was judged by an arbitrary scale based upon the recognition threshold, using the following designations: not present, just recognizable (threshold), slight, slight to moderate, moderate, moderate to strong, and strong (See Appendix C and D). Intensity of texture character notes

of juiciness and crumbliness was also judged by the arbitrary scale based upon the recognition threshhold (Appendix C). Intensity of chewiness was judged according to the number of chews required to masticate the sample at a constant rate of force application to reduce it to a consistency suitable for swallowing (Appendix E). Flavor profile notes were evaluated according to Appendix D and abbreviated texture profile notes were evaluated according to Appendix E.

Ground beef from each steer for flavor profile was packaged in 500 g portions and wrapped in freezer paper. The packages were frozen at -34°C. Within 4 weeks after ground beef formulation all samples were packed in dry ice and shipped by air to the Department of Food Science, North Carolina State University, at Raleigh for flavor profile evaluation. The samples were then stored at -28.9°C until the day before each panel evaluation.

Packages containing ground beef from one steer were randomly selected and thawed at room temperature. Six 110 ± 5 g patties approximately 2.5 cm thick were prepared, separated with waxed paper innerwrap, wrapped in freezer paper, and stored in a refrigerator until cooked. Ultra-fine gauge thermocouples (Omega Engineering, Inc., Stamford, Conn.) were inserted into the patty using a metal probe and cannula. Broiling temperature was monitored. Oven was allowed to preheat for 10 minutes and the oven door was left partially open during broiling. Patties were broiled approximately 15 cm from the heat source to an internal temperature of 71.1°C. Patties were turned once during cooking. Cooked patties were cut into 8 wedge shaped portions and each panelist was served 4 randomly selected portions of ground beef

from one steer in a sealed baby food jar for aroma and taste evaluation. Six panelists evaluated the samples at one time.

VI. VOLATILE ANALYSIS

Qualitative analysis of volatiles from a heated, lyophilized water extract of ground beef from 3 steers (one steer per Treatment 3, 4, and 5) were determined by a Tractor Model 222 GC interfaced with a Hewlett Packard mass spectrometer, Model No. 5930A, at 70 eV ionization potential and equipped with INCOS 2000 mass spectrometer data processing system (M.G. Legendre, personal communication). The GC column was 3m X 2.3 mm i.d. nickel packed with 10 percent Poly MPE on 60/80 mesh Tenax GC. The lyophilized extract was placed in a precolumn, heated to 150°C and held for 10 minutes under N_2 flow of 25 ml/min. Volatiles were concentrated on top of the Tenax GC column and were separated during a temperature programmed run from 80 to 220°C at 4 C/min.

The volatiles of lyophilized water extracts from each of 18 Hereford steers (6 per each Treatment 3, 4, and 5) were quantitated in a similar manner on a Bendix Model 2600 GLC equipped as previously described and adapted to the method described by Dupuy et al. (1977) except the precolumn was 7.5 cm X 6.3 mm o.d. and was placed in an oven specifically adapted to the Bendix GC. Ten mg of the lyophilized water extract were placed on volatile free glass wool in the precolumn and heated for 10 minutes at 150°C under a N_2 flow of 20 ml/min. Volatiles were collected at the start of a column previously described for the GC mass spectrometer but the packing was 7 percent Poly MPE instead of 10 percent. Volatiles were separated during a temperature programmed run from 80 to 220°C at 4 C/min.

VII. OTHER SELECTED ANALYSIS

Moisture and Fat Determination

Moisture and fat was determined on all the samples. Moisture was determined according to A.O.A.C. (1970), by drying the sample in a vacuum oven. Fat was determined by the anhydrous ethyl ether extraction method according to Ockerman (1969). Moisture and fat was determined in triplicate.

Color Measurements and Cooking Losses

Color and cooking losses were measured on ground beef from 18 Hereford steers finished by Treatment 3, 4, and 5 (6 trios).

One hundred twenty-five g patties prepared for color and moisture loss analysis were cooked for 3 minutes to an internal temperature of 68°C on a Presto Hamburger Cooker Model No. 05-MB2. The patties were cooled to room temperature. Color of raw and cooked patties were measured with a Hunterlab Color Difference Meter Model D-2520 which was standardized with the white calibrated standard No. C2-136 (L = +93.4, a = -1.1 and b = 1.9). Each patty was covered with plastic wrap and color was measured from two sides. The two values were averaged to give one reading. Each sample was done in duplicate for each raw and cooked patty. Cooking losses were calculated in duplicate as difference between raw and cooked weight of the patties.

VIII. ANALYSIS OF DATA

The data collected in this study were divided into four parts for analysis using the IBM 360/65 facilities at The University of Tennessee, Knoxville.

Data I consisted of characteristics of ground beef analyzed for Treatments 1, 2, 3, 4, and 5: fatty acid composition of neutral and polar lipids, taste panel flavor scores, water soluble simple carbohydrates, free amino compounds, moisture and fat content. In analysis of fatty acids, unknowns were grouped together by retention times. In Data I the effect of quintet and treatment were analyzed as shown in Table 3. When treatment source was found to be significant through regression analysis, orthogonal comparisons were used to find significant differences between means. Table 4 shows the comparisons. C1 analyzed means for significance due to type of ration (with the same energy level); C2 compared low energy ration to high energy ration; C3 analyzed means for significance due to season (summer vs winter); C4 compared silage and limited-grain fed to grain-fed during winter.

Additional data (Data II) were obtained for water-soluble carbohydrates and free amino compounds for Treatments 3, 4, and 5 at two additional frozen storage times, 6 and 12 months. The storage effect on these two characteristics was analyzed as shown in Table 5. Storage effects were tested for significance by Trio X Storage interaction and the Treatment X Storage interaction by the Trio X Storage X Treatment interaction. Significant storage effects were separated into linear and quadratic effects by orthogonal polynomials. When significant Treatment X Storage interactions were found, Storage means were separated for each treatment. If no significant Treatment X Storage interaction was found, storage means were separated across treatment.

Data III consisted of microbial numbers determined for Treatments 3, 4, and 5. In Data III the effect of Trio and Treatment were analyzed

TABLE 3

ANALYSIS OF VARIANCE FOR DATA I

Source	Degrees Freedom
Treatment	18
Quintet	4
Treatment X Quintet	72
Total	94

TABLE 4

ORTHOGONAL COMPARISON FOR DATA I AND II

	Treatment[a]				
Comparison	1	2	3	4	5
C1	0	0	+1	-1	0
C2	0	0	-1	-1	+2
C3	+3	+3	-2	-2	-2
C4	-1	+1	0	0	0

[a]1 = low energy, silage limited grain-fed, winter; 2 = high energy, grain-fed, winter; 3 = low energy, grass-fed, summer; 4 = low energy, limited grain-fed, summer; and 5 = high energy, grain fed, summer.

TABLE 5

ANALYSIS OF VARIANCE FOR DATA II

Source	Degrees of Freedom
Treatment	2
Trio	18
Treatment X Trio	36
Storage	2
Treatment X Storage	4
Trio X Storage	36
Trio X Storage X Treatment	72
Total	170

according to Table 6. Significant treatment means were separated by orthogonal comparisons C1 and C2 (Table 4).

Data IV consisted of characteristics studied on Treatments 3, 4, and 5 for ground beef from six Hereford steers: volatile analysis, color study, and cooking loss. In Data IV the effect of Trio and Treatment were analyzed according to Table 7. In case of unequal classes in analysis of variance means were adjusted. Significant treatment means were separated by orthogonal comparisons C1 and C2 (Table 4).

TABLE 6

ANALYSIS OF VARIANCE FOR DATA III

Source	Degrees of Freedom
Treatment	18
Trio	2
Treatment X Trio	36
Total	56

TABLE 7

ANALYSIS OF VARIANCE FOR DATA IV

Source	Degrees of Freedom
Treatment	5
Trio	2
Treatment X Trio	10
Total	17

CHAPTER IV

RESULTS AND DISCUSSION

I. GROUND BEEF COMPOSITION

Mean squares and treatment means for moisture and fat content of the ground beef prepared from each steer in Treatments 1 through 5 are presented in Tables 8 and 9. No treatment differences were found in moisture or fat content in the ground beef. The ground beef prepared from each steer in Treatments 1 through 5 had approximately 60 percent moisture and 19 percent fat.

II. MICROBIAL CONTAMINATION

Table 10 shows the mean squares for microbial numbers determined for Treatments 3, 4, and 5. The mean of microbial numbers of ground beef from the same treatments are shown in Table 11. Differences in microbial numbers were found for psychrophilic ($P < .01$) and lipolytic ($P < .05$) microorganisms due to ration energy level (C2, Table 10). Ground beef from steers in Treatments 3 and 4 (low energy levels) contained more psychrophilic and lipolytic microorganisms than that from steers on Treatment 5 (high energy level). This does not agree with Reagan et al. (1977) who determined microbial numbers for subcutaneous fat and lean tissue samples for beef ribs finished on different feeding regimes, and found that microbial numbers were not affected significantly by feeding regimes. However, considering that beef finished on low energy level rations had thinner subcutaneous fat (Cole, 1979) compared

TABLE 8

MEAN SQUARES FOR GROUND BEEF COMPOSITION

Source	DF	Moisture	Fat
Treatment	4	2.56	1.11
Quintet	18	2.48	1.12
Error (Q X T)	72	2.91	1.06

TABLE 9

TREATMENT MEANS OF GROUND BEEF COMPOSITION

Composition	Treatments[a]				
	1	2	3	4	5
	(%)				
Moisture	60.09	60.47	60.85	59.95	60.10
Fat	19.12	18.95	19.47	19.32	18.87

[a]1 = low energy, silage limited grain-fed, winter; 2 = high energy, grain-fed, winter; 3 = low energy, grass fed, summer; 4 = low energy, limited grain fed, summer; and 5 = high energy, grain fed, summer.

TABLE 10

MEAN SQUARES FOR MICROBIAL CONTENT OF GROUND BEEF

Source	DF	Aerobic	Psychrophilic	Proteolytic	Lipolytic
Trio	18	7591.19*	3377.29	2434.69**	7633.11
Treatment	2	1842.86	29860.02**	906.97	40845.26*
C1	1	970.11	10115.79	585.14	6187.43
C2	1	2715.62	49604.25***	1233.08	80073.39**
Error	36	3835.12	3902.59	788.42	8404.13

***$p < .001$.

**$p < .01$.

*$p < .05$.

TABLE 11

TREATMENT MEANS OF MICROBIAL CONTENT OF GROUND BEEF

Class	Treatment[a]		
	3	4	5
	1,000,000/g		
Aerobic	49.53	59.63	69.22
Psychrophilic	111.79	144.42	65.53
Proteolytic	51.40	43.21	35.87
Lipolytic	121.94	149.33	37.36

[a]3 = low energy, grass fed; 4 = low energy, limited grain fed; and 5 = high energy, grain fed.

to the beef finished on high energy level rations, larger areas of fat were needed for ground beef formulation for steers finished on low energy levels, since all ground beef samples contained 20 percent fat. Therefore, the increased number of psychrophilic and lipolytic micro-organisms in ground beef from steers finished on low energy levels, could be due to the larger area of fat.

III. SIMPLE CARBOHYDRATES AND FREE AMINO COMPOUNDS CONTENT

Table 12 shows mean squares from analysis of variance for simple carbohydrates and free amino compounds content of ground beef from Treatments 1, 2, 3, 4, and 5. The means of simple carbohydrates and free amino compounds for the 5 treatments are shown in Table 13. Table 14 shows the mean squares for the effect of storage on simple carbo-hydrates and free amino compounds in ground beef from Treatments 3, 4, and 5. The means of simple carbohydrates and free amino compounds content for Treatments 3, 4, and 5 during storage are shown in Table 15.

The amount of simple carbohydrates was lower in grass-fed beef samples than limited grain fed ($P < .01$) (C1, Table 12 and Table 13). This agrees with Brown et al. (1979) who reported the same result for a similar study. The low simple carbohydrates content of grass fed beef samples caused a difference ($P < .05$) when ground beef from low energy fed steers were compared with high energy fed steers as well as when summer fed steers were compared with winter fed steers (C2 and C3, Table 12 and Table 13). There was a decrease ($P < .001$) in the amount of simple carbohydrates during storage (Tables 14 and 15). When the sum of squares for the effect of storage on carbohydrates was par-titioned to show the type of variation, the linear and quadratic effects

TABLE 12

MEAN SQUARES FOR FREE SUGARS AND FREE AMINO COMPOUNDS

Source	DF	Free Sugars	Free Amino Compounds
Quintet	18	.08**	.0049*
Treatment	4	.20**	.0022
C1	1	.30**	.0030
C2	1	.23*	.0000
C3	1	.26*	.0013
C4	1	.00	.0044
Error	72	.04	.0026

** $p < .01$.

* $p < .05$.

TABLE 13

MEANS OF FREE SUGARS AND FREE AMINO COMPOUNDS OF GROUND BEEF BY TREATMENT

	Treatment[a]				
	1	2	3	4	5
Free sugars[b]					
mg glucose equiv./g meat	7.78	7.78	6.02	7.32	7.64
Free amino compounds[b]					
mg glycine equiv./g meat	1.74	1.75	1.72	1.79	1.76

[a]1 = low energy, silage limited grain-fed, winter; 2 = high energy, grain-fed, winter; 3 = low energy, grass fed, summer; 4 = low energy, limited grain fed, summer; and 5 = high energy, grain fed, summer.

[b]These are reported on as is basis.

TABLE 14

MEAN SQUARES FOR FREE SUGARS AND FREE AMINO COMPOUNDS OF GROUND BEEF

Source	DF	Free Amino Compounds	Free Sugars
Treatment	2	.036	29.104**
Trio	18	.504*	5.810
Treatment X Trio	36	.299	4.900***
Storage	2	15.525***	77.362
Linear	1	29.970***	139.332***
Quadratic	1	.125	18.727***
Treatment X Storage	4	.238	.275
Trio X Storage	36	.410**	1.859
Residual	63	.201	1.386
Total	161	.481	4.198

***$p < .001$.

**$p < .01$.

*$p < .05$.

TABLE 15

SIMPLE CARBOHYDRATES AND FREE AMINO COMPOUNDS CONTENT FOR THREE TREATMENTS AND THREE STORAGE PERIODS

	Treatments[a]		
	3	4	5
	mg/g meat (wet basis)[b]		
Simple carbohydrates, as glucose			
0 month	6.02	7.31	7.65
6 month	4.44	5.36	5.65
12 month	3.91	4.86	5.59
Free amino compounds, as glycine			
0 month	1.72	1.79	1.76
6 month	2.41	2.18	2.43
12 month	2.76	2.89	2.70

[a]3 = low energy, grass fed, summer; 4 = low energy, limited grain fed, summer; and 5 = high energy, grain fed, summer.

[b]Means of three determinations for all the samples in the group.

were significant. The decrease in simple carbohydrates during storage does not agree with Brown et al. (1979) who found a significant increase in the amount of simple carbohydrates during 5 months frozen storage. The reason why is not known.

There was not a significant difference in the free amino compounds content due to ration or level of energy (Tables 12 and 13). Free amino compounds content increased ($P < .001$) during storage (Tables 14 and 15). When the sum of squares for the effect of storage was partitioned to show the type of variations, only the linear effect was significant. Studies have indicated that during refrigerator storage free amino acids increase (Gardner and Stewart, 1966; Locker, 1960; Saffle et al., 1961). The results for free amino compounds among treatments and during storage also agree with Brown et al. (1979) who found no significant difference in amino compounds among steers due to ration or energy level but found a significant increase in amino compounds during 5 months frozen storage.

IV. FATTY ACID COMPOSITION

Mean squares from analysis of variance for fatty acid composition of neutral and polar lipids are shown in Tables 16 and 17. Mean percentage values for the fatty acids found in neutral and polar lipids for each treatment are shown in Tables 18 and 19.

In the neutral lipid fraction, there were significant differences in the relative percentages of myristic (C14:0), myristoleic (C14:1), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids. Orthogonal comparisons showed the differences among treatments. During winter, steers fed a

TABLE 16

MEAN SQUARES FOR FATTY ACIDS OF NEUTRAL LIPIDS

		Fatty Acids							
Source	DF	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Quintet	18	.33**	.34	1.42	.25	5.15	167.38	.34	.04
Treatment	4	.46*	1.13**	9.64**	1.08**	53.39***	121.58***	7.48***	1.75***
C1	1	.39	1.65**	22.48**	.47	4.26	1.04	.85	3.74***
C2	1	1.04*	2.60**	.40	.98	110.15***	280.64***	.49	3.10***
C3	1	.12	.07	11.21*	2.64**	89.94***	129.09***	17.93***	.01
C4	1	.17	.05	4.68	.01	4.61	76.35*	11.13***	.12
Error	68	.15	.20	2.21	.21	4.55	72.98	.49	.04

***$p < .001$.

**$p < .01$.

*$p < .05$.

TABLE 17

MEAN SQUARES FOR FATTY ACIDS OF POLAR LIPIDS

Source	DF	Fatty acids														
		C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C22:0	C20:4	X	Y	Z
Quintet	18	1.16	.78	3.22	15.40	23.87	1.82	8.72	10.88	11.08	.14	.91	5.29	.89	1.12	1.66
Treatment	4	3.56*	.82	6.09	19.36	22.27	3.76	40.29***	12.30	10.78	4.33***	1.73	10.13	6.82***	4.33**	6.38*
C1	1	.17	.12	.60	2.39	12.39	1.17	3.14	2.49	7.62	10.66***	.09	27.11*	.02	2.54	.54
C2	1	.81	.51	1.26	.33	72.55	4.14	66.22**	15.31	2.72	6.63**	4.23*	7.61	.02	7.26**	1.27
C3	1	6.27*	2.11	22.26**	70.06*	2.53	9.70*	91.20**	1.30	.02	.02	1.30	5.68	25.72***	6.84*	15.31**
C4	1	6.97*	.53	.24	4.68	1.61	.04	.61	30.11	32.76*	.00	1.30	.13	1.52	.69	8.39*
Error	68	1.05	.67	2.83	12.61	37.06	1.93	5.89	19.57	6.47	.47	1.01	4.18	.90	1.02	1.87

*** P < .001
** P < .01
* P < .05

TABLE 18

MEAN FATTY ACID COMPOSITION OF NEUTRAL LIPIDS

Fatty Acid	Treatments[a]				
	1	2	3	4	5
	(%)				
C14:0	2.43	2.57	2.62	2.42	2.24
C14:1	1.45	1.53	1.80	1.38	1.14
C16:0	26.07	26.83	26.33	27.87	27.28
C16:1	3.69	3.68	3.54	3.32	3.15
C18:0	13.36	12.58	15.64	16.31	13.03
C18:1	51.91	49.01	46.30	46.63	51.18
C18:2	1.87	2.97	1.73	1.43	1.39
C18:3	.96	.84	1.40	.77	.59

[a]1 = low energy, silage limited grain-fed, winter; 2 = high energy, grain fed, winter; 3 = low energy, grass fed, summer; 4 = low energy, limited grain fed, summer; and 5 = high energy, grain fed, summer.

TABLE 19

MEAN FATTY ACID COMPOSITION OF POLAR LIPIDS

Fatty Acid	Treatments[a]				
	1	2	3	4	5
	(%)				
C14:0	1.74	2.65	1.66	1.79	1.47
C14:1	.49	.74	.32	.43	.17
C15:0	3.43	3.70	2.30	2.55	2.74
C16:0	23.07	23.65	21.68	21.18	21.59
C16:1	4.90	4.50	4.50	5.65	2.68
C17:0	1.40	1.35	2.46	2.10	1.71
C18:0	12.49	12.51	15.09	15.66	13.09
C18:1	35.48	33.53	34.09	33.58	34.94
C18:2	6.95	9.00	7.44	8.34	8.35
C18:3	.93	.92	1.72	.66	.47
C22:0	1.52	1.16	1.36	1.45	1.98
C20:4	3.97	4.20	3.46	5.15	5.08
X	1.09	.70	2.01	1.97	1.95
Y	1.00	.75	.90	1.42	1.92
Z	1.72	.79	2.09	2.33	1.89

[a]1 = low energy, silage limited grain-fed, winter; 2 = high energy, grain fed, winter; 3 = low energy, grass fed, summer; 4 = low energy, limited grain fed, summer; and 5 = high energy, grain fed, summer.

low energy ration (Treatment 1) had higher C18:1 and lower C18:2 than steers fed the high energy ration (Treatment 2). Steers fed during winter (Treatments 1 and 2) had less C16:0 and C18:0 and more C16:1, C18:1 and C18:2 than summer-fed steers (Treatments 3, 4, and 5). During summer, grass fed steers (Treatment 3) had lower C16:0 and higher C14:1 and C18:3 than grain-fed steers (Treatment 4). Steers fed low energy rations during summer (Treatments 3 and 4) had lower C18:1, and higher C14:0, C14:1, C18:0 and C18:3 than steers fed a high energy ration (Treatment 5).

In the polar lipids there were significant differences in the relative percentages of myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0), linoleic (C18:2), linolenic (C18:3), erucic (C22:0), arachidonic (C20:4), and unknowns X, Y and Z. It was not established whether unidentified peaks belonged to fatty acids or their oxidation products. Orthogonal comparisons showed the differences among treatment means. Steers fed silage and limited grain during winter (Treatment 1) had lower C14:0 and C18:2 and higher unknown Z than steers fed full grain (Treatment 2). Winter-fed steers (Treatments 1 and 2) had lower C17:0, C18:0 and unknowns X, Y, and Z than summer-fed steers (Treatments 3, 4, and 5). Steers fed grass during summer (Treatment 3) had higher C18:3 and lower C20:4 than steers fed limited grain (Treatment 4). Steers fed the high energy, full grain ration during summer (Treatment 5) had lower C18:0 and C18:3 and higher C22:0 and unknown Y than low energy fed steers (Treatments 3 and 4).

The fatty acid composition of neutral and polar lipids in steers on Treatments 3, 4, and 5 agrees fairly well with results published by

Brown et al. (1979). Grass-fed steers had higher C18:3 in neutral and polar lipids, respectively. Ground beef from grass-fed steers in the present study had 1.40 percent and 1.72 percent C18:3 in neutral and polar lipids, respectively (Tables 18 and 19). Westerling and Hedrick (1979) reported that steers and heifers which were finished on predominantly fescue grass had higher C18:3 (0.79 percent) in intramuscular fat than steers and heifers finished on corn ration for 56 or 112 days. These investigators extracted lipids by anhydrous ether which most likely did not extract bound phospholipids which contain more C18:3 than neutral lipids. Also, it is difficult from their description of materials and methods to tell if lipid extracts were protected from oxidation which could reduce C18:3 content further.

In contrast with Brown et al. (1979) who found that neutral and polar lipids of grass-fed steers had less C18:2 and Westerling and Hedrick (1979) who reported that subcutaneous fat of grass-fed cattle had less C18:2 than grain-fed cattle, steers fed grass during the summer in this present investigation did not have less C18:2 in neutral and polar lipids. However, steers fed a low energy ration during the winter had less C18:2 in neutral and polar lipids than steers fed a high energy ration (Tables 18 and 19).

V. SENSORY EVALUATION

Taste Panel

Analysis of variance and treatment means for flavor scores of ground beef prepared from each steer in Treatments 1 through 5 are shown in Tables 20 and 21. Significant differences among treatments were found. Ground beef from summer, grass-fed steers (Treatment 3)

TABLE 20

ANALYSIS OF VARIANCE FOR FLAVOR SCORES OF GROUND BEEF

Source	DF	SS	MS
Quintet	18	7.56	.42*
Treatment	4	31.88	7.97***
C1	1	3.12	13.12***
C2	1	7.48	7.48***
C3	1	11.23	11.23***
C4	1	.06	.06***
Error	72	15.12	.21

***$p < .001$.

*$p < .05$.

TABLE 21

MEANS OF FLAVOR SCORE OF GROUND BEEF BY TREATMENT

	Treatment[a]				
	1	2	3	4	5
Flavor Score[b]	6.55	6.47	4.96	6.14	6.32

[a] 1 = low energy, silage limited grain-fed, winter; 2 = high energy, grain-fed, winter; 3 = low energy, grass fed, summer; 4 = low energy, limited grain fed, summer; and 5 = high energy, grain fed, summer.

[b] 1 = extremely undesirable, 9 = extremely desirable.

had a lower flavor score than ground beef from summer limited grain fed steers (Treatment 4). The difference between Treatment 3 and 4 was great enough to cause a significant difference between low energy rations and the high energy ration in summer (C2, Table 20). Ground beef from winter fed steers (Treatment 1 and 2) had a higher flavor score than summer fed steers. This difference is related, in part, to the low flavor score of grass fed steers (Treatment 3). The finding that ground beef from grass-fed steers had less desirable flavor agrees with several researchers who documented less desirable flavor in grass-fed beef (Brown et al., 1979; Dinius and Cross, 1978; Reagan et al., 1977; Skelly et al., 1978; Westerling and Hedrick, 1979).

Flavor Profile

The flavor profile panel at North Carolina State University found that there was a dairy or milky flavor in grass fed beef which at higher intensities was particularly unpleasant. They defined the dairy flavor as a pleasant, sweet, creamy, buttery odor characteristic of warm, freshly drawn milk and associated with dairy facilities, milk pails and baby bottles; an odor which is also perceived upon entering a cooler containing chilled hanging beef carcasses (T. N. Blumer, personal communication). Figure 1 shows histograms for 18 Hereford steers, 6 steers per treatment 3 (grass-fed), 4 (limited grain-fed) and 5 (grain-fed) in which the intensity of the dairy flavor and aftertaste and a beef fat flavor are presented for each steer. Of all flavor notes determined by the flavor profile panel, only 3 attributes showed differences which might be correlated with inferior flavor scores of grass-fed beef compared with grain-fed beef (Table 21).

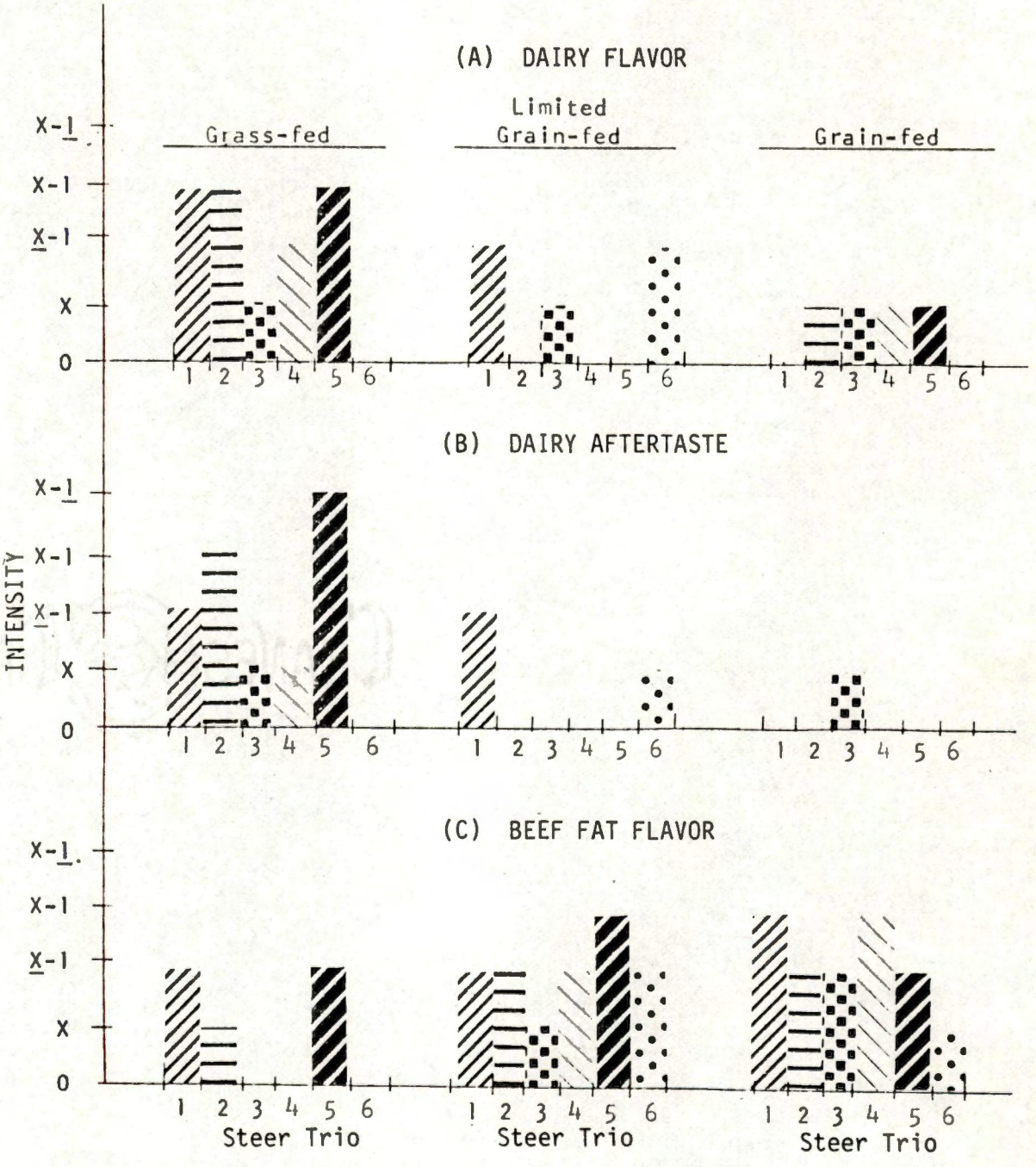

Figure 1. Histograms of Some Flavor Character Notes of Broiled Ground Beef Patties Determined by Flavor Profile Panel (0 = Not Present, X = Just Recognizable, 1 = Slight).

The dairy flavor and aftertaste in trios 1, 2, 3, 4, and 5 generally are higher intensity in grass-fed steers than in limited grain-fed or grain-fed steers (Figure 1). Also, grass-fed steers had a lower intensity of a beef fat flavor which the panel defined as the aromatic flavor of freshly cooked beef fat. It is possible that the higher intensity of the dairy flavor and lower intensity of the beef fat flavor are the causes of the less desirable grass-fed beef flavor. However, it is not possible to statistically analyze flavor profile intensity results; therefore, only possible relationships between intensity of character notes and the flavor score may be noted.

VI. COLOR MEASUREMENT AND COOKING LOSS

Tables 22 and 23 show the analysis of variance for Hunter color parameters of "L," "a," and "b" for raw and cooked patties of ground beef from Treatments 3, 4, and 5. Means of three color parameters are shown in Table 24. Differences ($P < .05$) were found among treatments for color parameters of lightness (L) and redness (a) in raw patties. Raw patties from grain-fed steers had lower "L" ($P < .01$) and higher "a" ($P < .05$) than low energy fed steers (Table 24). No significant difference was found for yellowness (b). These data do not match the Malphrus (1961) and Gann (1977) data who analyzed the fat color from steers on different feeding managements and found that fat of pasture fed steers is more yellow than drylot steers. Probably the color of fat is somehow masked in ground beef by differences in lean color. In cooked patties significant treatment difference were found for lightness (L) (C2, Table 23). Cooked patties from high energy, grain-fed steers had lower "L" ($P < .05$) than low energy fed steers (Table 24).

TABLE 22

ANALYSIS OF VARIANCE OF "L,"[a] "a"[a] AND "b"[a] COLOR PARAMETERS OF RAW BEEF PATTIES

Source	DF	MS	F
Parameter "L"			
Trio	5	5.858	.34
Treatment	2	38.111	5.50
C1	1	.083	.02*
C2	1	38.028	10.98**
Error	10	34.636	
Parameter "a"			
Trio	5	2.873	.87
Treatment	2	5.693	4.29
C1	1	.403	.61*
C2	1	5.290	7.97*
Error	10	6.633	
Parameter "b"			
Trio	5	1.318	1.81
Treatment	2	.274	.94
C1	1	.007	.05
C2	1	.267	1.84
Error	10		

[a] L = lightness, a = redness^{+}, gray 0, green^{-}, b = yellow^{+}, gray 0, blueness^{-}, in Hunter color.

**$p < .01$.

*$p < .05$.

TABLE 23

ANALYSIS OF VARIANCE OF "L,"[a] "a"[a] AND "b"[a] COLOR PARAMETERS OF COOKED BEEF PATTIES

Source	DF	MS	F
Parameter "L"			
Trio	5	51.218	2.07
Treatment	2	30.854	3.11
C1	1	1.333	.27
C2	1	29.521	5.96*
Error	10		
Parameter "a"			
Trio	5	1.331	1.26
Treatment	2	.781	1.84
C1	1	.001	.00
C2	1	.780	3.68
Error	10		
Parameter "b"			
Trio	5	0.744	0.29
Treatment	2	1.174	1.13
C1	1	.001	.00
C2	1	1.173	2.26
Error	10		

[a]L = lightness, a = redness^{+}, gray 0, green^{-}, b = yellow^{+}, gray 0, blueness^{-}, in Hunter color.

*P < .05.

TABLE 24

HUNTER COLOR VALUES FOR RAW AND COOKED GROUND BEEF PATTIES FROM TREATMENT 3, 4, AND 5

Treatment[a]	L[b]	a[b]	b[b]
Raw 3	41.867	7.717	11.467
4	41.700	8.083	11.417
5	38.700	9.050	11.183
Cooked			
3	44.517	2.883	11.400
4	43.850	2.867	11.417
5	41.467	3.317	11.950

[a]3 = Low energy, grass-fed, summer; 4 = low energy, limited grain-fed, summer; and 5 = high energy, grain fed, summer.

[b]L = lightness, a = redness^{+}, gray 0, green^{-}; b = yellow^{+}, gray 0, blueness^{-}, in Hunter color.

No significant differences were found for redness (a) or yellowness (b). Darker color of cooked patties from grain-fed steers could be due to the darker color of the raw ground beef patties from grain-fed steers as well as higher simple carbohydrate content which causes a higher rate of browning reaction.

Analysis of variance for cooking loss of ground beef from steers on Treatments 3, 4, and 5 along with orthogonal comparisons are shown in Table 25 and Treatment means in Table 26. No significant differences were found. Data show feeding management does not effect cooking loss of ground beef patties.

VII. VOLATILE ANALYSIS

Volatiles from a heated, lyophilized water extract of ground beef from a grass-fed steer are identified in Figure 2. No significant qualitative or quantitative differences were found in volatiles between grass-fed and grain-fed steers (Table 27). Most likely the actual volatile compounds which cause the less desirable flavor in grass-fed beef are fat soluble.

VIII. RELATIONSHIP OF FLAVOR SCORE AND CHEMICAL CHARACTERISTICS

Table 28 shows correlation coefficients between flavor score and chemical components of ground beef. Partial regression coefficients and R^2 for regression models predicting flavor score from chemical composition are shown in Table 29. The data show several chemical components are correlated significantly with flavor. One chemical component which could contribute to desirable flavor is the water-soluble free sugars with a correlation coefficient of .35 and which accounted

TABLE 25

ANALYSIS OF VARIANCE FOR COOKING LOSS OF GROUND BEEF PATTIES

	DF	MS	F[a]
Trio	5	61.271	.83 ns
Treatment	2	42.074	1.42 ns
C1	1	39.241	2.66 ns
C2	1	2.834	.19 ns
Error	10	147.746	
Total	17	251.091	

[a]"ns" means not significant at the 5% level.

TABLE 26

COOKING LOSS OF GROUND BEEF PATTIES FROM TREATMENT 3, 4, AND 5

	Treatment[a]		
	3	4	5
	(%)		
Cooking Loss	30.30	33.21	32.45

[a]3 = Low energy, grass-fed, summer; 4 = low energy, limited grain-fed, summer; and 5 = high energy grain-fed, summer.

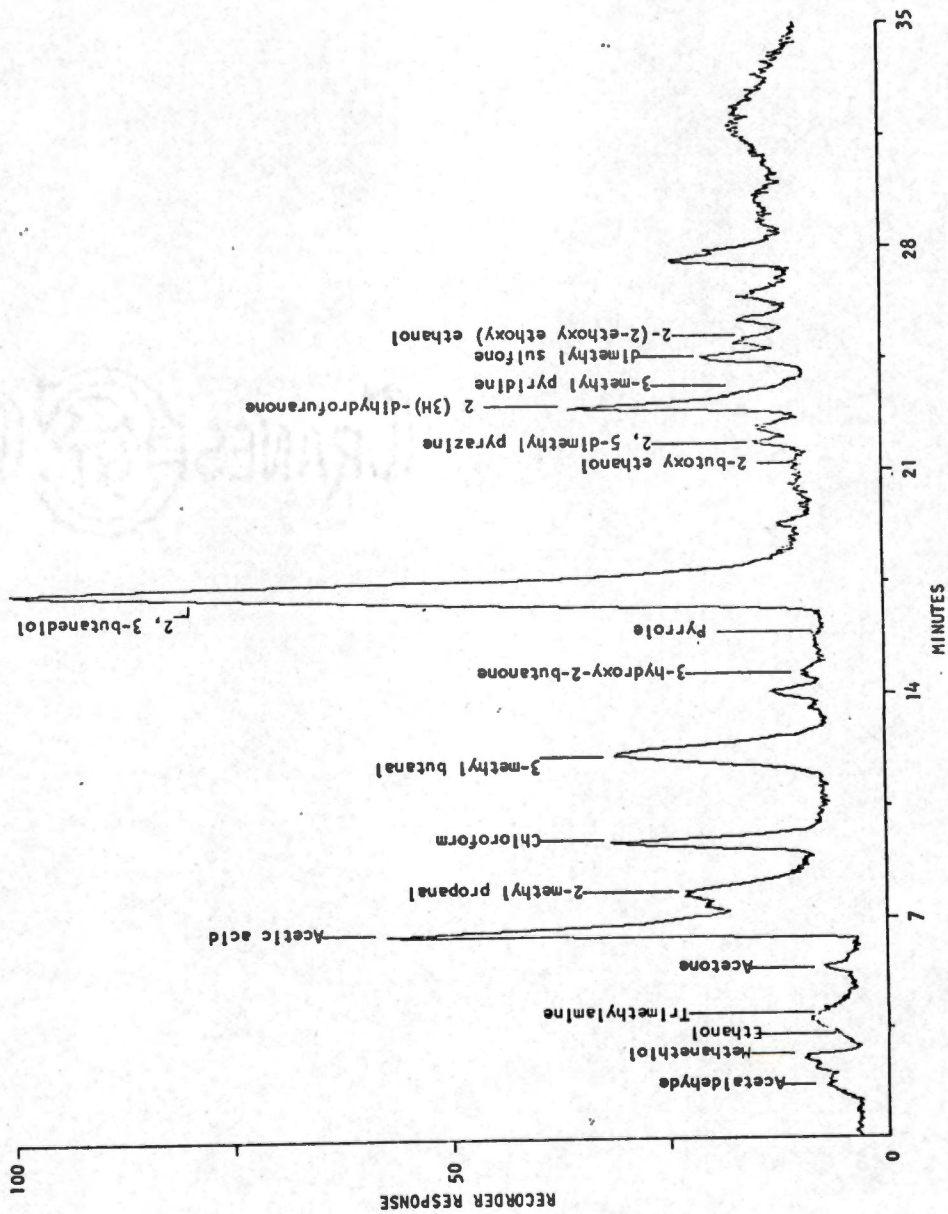

Figure 2. Chromatogram of Volatiles from a Heated Lyophilized Water Extract of Ground Beef from a Grass-Fed Steer.

TABLE 27

TREATMENT MEAN SQUARES AND "F" VALUES FOR VOLATILES OF HEATED LYOPHILIZED WATER EXTRACT OF GROUND BEEF FROM TREATMENTS 3, 4 AND 5

Volatile	MS	F[a]
Methanethiol	.336	.76 ns
Ethanol	.465	.62 ns
Acetone	.25	.32 ns
Acetic acid and 2-methyl propanal	5.535	.98 ns
Chloroform	.174	.35 ns
3-methyl butanal	.780	1.72 ns
3-hydroxy-2-butanone	.311	.52 ns
Pyrrole	.471	.58 ns
2,3-butanediol	175.102	2.20 ns
2-butoxy ethanol	4.272	1.49 ns
2,5-dimethyl pyrazine	.342	.27 ns
2(3H)-dihydrofuranone	.410	.09 ns
3-methyl pyridine and dimethyl sulfone	1.202	.78 ns
2(2-ethoxy ethoxy) ethanol	1.335	.27 ns

[a] "ns" means not significant at 5% level.

TABLE 28

CORRELATION COEFFICIENTS BETWEEN FLAVOR SCORE AND CHEMICAL COMPONENTS OF GROUND BEEF

Component	Source of Chemical Component		
	Neutral Lipids	Polar Lipids	Water Solubles
	r		
Fatty Acids	-.16	.06	--
C14:0	-.31**	.05	--
C14:1	--	.19	--
C15:0	.09	.02	--
C16:0	-.03	.01	--
C16:1	--	-.14	--
C17:0	-.25*	-.18	--
C18:0	.27**	.06	--
C18:1	.13	.08	--
C18:2	-.49***	-.40***	--
C18:3	--	.05	--
C22:0	--	.11	--
C20:4	--	-.32**	--
X	--	.04	--
Y unknowns	--	-.18	--
Z	--		--
Free sugars			.36
Free amino compounds			.07

***$p < .001$.

**$p < .01$.

*$p < .05$.

TABLE 29

PARTIAL REGRESSION COEFFICIENTS AND R^2 FOR REGRESSION MODELS PREDICTING FLAVOR SCORE FROM CHEMICAL COMPOSITION

Items in Model	Flavor Score: Partial Regression Coefficient[a]	Flavor Score: R^2[b]
Intercept	-1.865	-
Neutral Lipids		
C14:0	.191	.025
C14:1	- .375	.098**
C16:0	.095	.008
C16:1	.245	.001
C18:0	.063	.060*
C18:1	.054	.072**
C18:2	.046	.018
C18:3	- .285	.244***
Polar Lipids		
C14:0	- .096	.003
C14:1	.053	.002
C15:0	.065	.035
C16:0	- .021	.001
C16:1	.030	.000
C17:0	.066	.020
C18:0	- .001	.032
C18:1	.017	.004
C18:2	.026	.006
C18:3	- .211	.156***
C22:0	.383*	.003
C20:4	- .096	.011
X	- .353*	.100**
Y unknowns	.105	.002
Z	- .183	.031
Water Soluble		
Free sugars	.118	.129***
Amino compounds	.034	.005
All above items	-	.592***
All neutral fatty acids	-	.340***
All polar fatty acids	-	.464***
All water solubles	-	.139***

[a]When all items are included in model.

[b]For each item alone and/or in combination with other items in a model.

***$p < .001$.

**$p < .01$.

*$p < .05$.

for 12.9% of the variation in the flavor score. In neutral lipids, C14:1, C18:0, C18:1 and C18:3 were significantly correlated to flavor score. C18:1 had a positive correlation coefficient while C18:3 had the lowest negative correlation coefficient. Fatty acids of neutral lipids accounted for 34.0% of variation in the flavor score (Table 29). In polar lipids, C18:3 and one of the unknowns were negatively correlated with flavor score, total fatty acids of polar lipid was responsible for 46.4 percent of the variation in flavor score. When fatty acid composition of polar and neutral lipids and water soluble free sugars and amino compounds all were considered in a regression model to predict flavor score, they accounted for 59.2 percent of the variation (Table 29).

The high R^2 for regression model predicting flavor score from fatty acid composition of neutral or polar lipids, and the lack of any qualitative or quantitative differences between volatiles from a heated, lyophilized water extract of ground beef between grass-fed and grain-fed steers, show most likely the compounds responsible for off-flavor in grass fed beef are fat soluble or are formed in fat during heating. It is possible that the higher C18:3 content and the higher bacterial contamination of the ground beef from grass-fed steers contributed to a higher intensity of a dairy flavor. Wong et al. (1975) suggested that certain organisms can produce, from long chain acids, shorter chain hydroxy fatty acids which could lactonize. They found that longer chain lactones such as δ-C14 and δ-C16 contribute to rancidity in cheddar cheese. If C18:3 were the substrate of a microorganism, there is a possibility that an unsaturated lactone might form. Several unsaturated lactones such as 6-isobutyl-α-pyrone or other lactones

such as 2-deceno-δ-lactone have been characterized as having a dairy, creamy, buttery or milky aroma and flavor (Nobuhara, 1968; Pittet and Klaiber, 1975). These lactones at low concentrations are desirable in synthetic butter but become undesirable and unpleasant at higher concentrations (Urback et al., 1972). More research is needed to isolate the actual flavor volatile(s) responsible for the dairy or milky flavor in grass-fed beef.

CHAPTER V

SUMMARY

The purpose of this study was an investigation of selected chemical characteristics, changes in some of the chemical characteristics as a function of frozen storage time, isolation and identification of flavor volatiles and subjective evaluation of flavor of frozen ground beef from steers finished on forage and grain rations.

For this study 95 steers were grouped into 19 quintets on the basis of breed, weight and body type. One steer of each quintet was finished on a silage-limited grain ration (T1) and another of each quintet was finished on a grain ration during the 1978 winter (T2). The other three steers were wintered on pasture and in April 1978, one of each quintet was finished either on orchard grass, fescue and clover pasture (T3), a limited grain ration (T4) or a full grain ration (T5) during the 1978 summer.

Ground beef containing approximately 20 percent fat was prepared from the semimembranosus muscle and brisket fat of the left side of each carcass. Simple carbohydrates, free amino compounds, fatty acid composition of neutral and polar lipids, flavor score, moisture and fat content were determined for ground beef prepared from steers in T1 through T5. Bacterial count was determined for ground beef prepared from T3, T4, and T5. Changes in simple sugar content and free amino compounds were studied as a function of frozen storage time on T3, T4, and T5. Flavor profile, volatile analysis of heated lyophilized water extract and Hunter color for raw and cooked patties were studied for ground beef prepared from 18 Hereford steers, 6 each for T3, T4, and T5.

No significant difference was found for moisture and fat content among treatments. Ground beef from the low energy level T3 and T4 had a higher population of psychrophilic and lipolytic bacteria than ground beef from high energy T5. Ground beef from summer, grass-fed steers (T3) had less sugar, lower flavor score and higher linolenic acid in neutral and polar lipids. Ground beef from grass-fed steers had also lower C18:1 and C20:4, and higher C14:0, C14:1 and C18:0. Steers fed during winter had less C16:0 and C18:0 and more C16:1, C18:1, and C18:2 than summer-fed steers. Other differences in the fatty acid composition of neutral and polar lipids among treatments were also observed. No significant differences among treatments were found for free amino compounds. There was an increase in free amino compounds and a decrease in simple sugar content during frozen storage time.

A dairy or milky flavor which was observed by the flavor profile panel in grass-fed beef was particularly unpleasant at higher intensities. Also, there was a lack of beef fat flavor in grass-fed beef. No significant qualitative or quantitative differences were found between volatiles of heated lyophilized water extract of ground beef prepared from grass-fed steers and that of grain-fed steers.

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APPENDICES

APPENDIX A

UNIVERSITY OF TENNESSEE BULL TEST RATION

Corn, #2, yellow shelled	59%
Cottonseed meal 41% protein or Soybean meal 44%	10%
Molasses, cane	5%
Dehydrated alfalfa meal	3%
Animal fat	2%
Ground limestone	.5%
Salt	.5%
Cottonseed hulls coarsely ground	20%

APPENDIX B

Name _____ Date _____

PLEASE READ INSTRUCTION BEFORE TASTING.

You will be given 5 samples of ground beef patties to smell and taste for flavor.

MAKE SURE YOU SMELL THE PATTY FIRST, THEN TASTE the entire portion which is served before you make up your mind. You do not have to swallow the sample. You can expectorate in the empty cup provided.

Rinse your mouth thoroughly with the water provided between samples.

Use the scale below to indicate your attitude to the overall flavor of each patty and check at the point on the scale which best describes your feeling about each ground beef patty. Also your comments would be appreciated.

	Code	Code	Code	Code	Code
	____	____	____	____	____
Extremely desirable	____	____	____	____	____
Desirable	____	____	____	____	____
Moderately desirable	____	____	____	____	____
Slightly desirable	____	____	____	____	____
Acceptable	____	____	____	____	____
Slightly undesirable	____	____	____	____	____
Moderately undesirable	____	____	____	____	____
Undesirable	____	____	____	____	____
Extremely undesirable	____	____	____	____	____
	Comments	Comments	Comments	Comments	Comments

APPENDIX C

Intensity Scale		Code
0	not present	1
X	just recognizable, threshold	2
X-1		3
X-1		4
X-1		5
1	slight	6
1-2		7
1-2	slight to moderate	8
1-2		9
2	moderate	10
2-3		11
2-3	moderate to strong	12
2-3		13
3	strong	14

APPENDIX D

GROUND BEEF FLAVOR PROFILE DEFINITIONS

Aroma: Sensations perceived by the nose when the sample is sniffed. All samples are evaluated for aroma prior to flavor evaluation.

Cooked beef: characteristic odor of warm, freshly-cooked beef.
Dairy: a pleasant, sweet, creamy, buttery odor characteristic of warm, freshly-drawn milk and associated with dairy facilities, milk pails, and baby bottles; an odor which is also perceived upon entering a cooler containing chilled hanging beef carcasses.
Briny: aroma of saline solutions.
Sweet: a general aroma character associated with products that have a sweet basic taste.
Sour: a general aroma character associated with products that have a sour basic taste.
Beef fat: odor of freshly-cooked beef fat.
Green: a dusty, woody odor associated with freshly-cut alfalfa, green hay, or green grasses.
Browned: a seared meat odor.

Flavor: Sensations perceived by the tongue, mouth surfaces, throat, and nose when the sample is eaten. Flavor includes the basic tastes, odors in the nose (aromatics), and feeling factors in the mouth.

Beef: aromatic flavor characteristic of warm, freshly-cooked beef.
Salt: the basic taste.
Sour: the basic taste.
Sweet: the aromatic character associated with products that have a sweet basic taste.
Fat mouthfeel: a coating of fat on mouth surfaces, particularly noticeable on the palate.
Blood-like: aromatic flavor suggestive of blood.
Metallic: mouthfeel typically resulting from holding aluminum foil or a copper penny in the mouth.
Dairy: aromatic flavor associated with the dairy aroma defined above.
Bitter: the basic taste.
Fat: aromatic flavor of beef fat.
Liver: aromatic flavor suggestive of liver.
Astringent: quality perceived through the complex of sensations caused by shrinking, drawing, or puckering of the skin surfaces of the oral cavity; a dry feeling in the mouth.
Green: aromatic flavor associated with the green aroma defined above.
Browned: aromatic flavor associated with the browned aroma defined above.
Soured dairy: unpleasant aromatic flavor associated with soured milk and resembling regurgitated baby's milk.
Stale: aromatic flavor suggestive of prolonged storage, as if the sample is "old".
Putrid: aromatic flavor associated with proteolytic spoilage.

Aftertaste: Flavor sensations perceived by the tongue, mouth surfaces, throat, and nose after the sample is swallowed.

Sour: the basic taste.
Beef: aromatic as defined above.
Blood-like: aromatic as defined above.
Dairy: aromatic as defined above.
Fat mouthfeel: mouthcoating as defined above.
Astringent: mouthfeel as defined above.
Metallic: mouthfeel as defined above.
Bitter: the basic taste.

Additional character notes which were detected in only one or two samples are defined as follows:

Aroma:

Earthy green: a damp, moldy, earthy odor characteristic of roots and attached soil, and resembling the odor of green (raw) peanuts and peanut hulls.

Wet manure: initial impression is a sweet, floral, perfumy aromatic odor, but as aroma lingers in the nose, it becomes very unpleasant; the odor is suggestive of wet manure (possibly chicken) and resembles the aroma of scalded chicken feathers.

Dirty socks: odor usually associated with dirty, smelly, sweaty feet, socks, and tennis shoes.

Flavor:

Earthy green: aromatic flavor associated with the earthy green aroma defined above.

Aftertaste:

Earthy green: aromatic as defined above.

Fat: aromatic flavor of beef fat.

APPENDIX E

GROUND BEEF ABBREVIATED TEXTURE PROFILE

Name: _____ Date: _____

Sample number				
A. Juiciness				
Crumbliness				
B. Chewiness				

Techniques and terminology:

The masticatory phase of a texture profile encompasses mechanical, geometrical, and moisture and fat characteristics which are perceived during chewing. For this abbreviated texture profile of ground beef, only the most obvious characteristics of the product are evaluated; all are during the masticatory phase.

A. Place bite-size sample (approximately one-eighth of a ground beef patty, pie-shaped to include interior and exterior of the patty) between molar teeth and, biting down evenly and at a constant rate, masticate up to 10 chews and evaluate the following:

Juiciness: amount of meat juices and oils released during chewing. This characteristic is a mouthfeel quality and is related to the moisture and fat content of the sample.

Crumbliness: ease with which the sample crumbles. This secondary mechanical characteristic is a combination of the parameters of hardness and cohesiveness; samples which are crumbly possess low resistance to force (low hardness) and very low deformation before fracturing (low cohesiveness).

B. Masticate sample at a rate of 1 chew per second at an applied force equal to that required to penetrate a gum-drop in a half-second and evaluate the following:

Chewiness: number of chews required to masticate the sample at a constant rate of force application to reduce it to a consistency suitable for swallowing. Chewiness is also a secondary mechanical characteristic and is a combination of the parameters of hardness, cohesiveness, and elasticity.

VITA

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