Dietary Influence on Lipid Composition and Oxidation of Fresh and Processed Meat Products

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Kaitlyn Margaret Compart

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Ryan B. Cox, Adviser
Alfredo DiCostanzo, Co-Adviser

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Dedication

This work is dedicated to my family and my husband, David. Nothing is possible without them.
Ní ceart go cur le chéile.

Abstract

Two experiments were conducted to evaluate the effects of diet on fresh and processed meat quality in beef. In experiment 1, steers and heifers (n = 48) were assigned randomly to one of four treatment groups and fed individually. Treatments were as follows: steam-flaked corn diet with no modified distillers grains with solubles (MDGS) or glycerin (CON); CON with 35% MDGS (MDGS); CON with 10% glycerin (GLY); and CON with 35% MDGS and 10% glycerin (MDGS/GLY). When cattle reached a mean weight of 590 kg, they were humanely harvested at a commercial abattoir. Strip loins and shoulder clods were removed from the right side of each carcass. Treatment had no effect any specific fatty acid ($P > 0.05$), vacuum purge loss ($P = 0.75$), cooking loss ($P = 0.40$), Warner-Bratzler shear force values ($P = 0.94$), strip steak L*, a*, or b* values ($P > 0.05$) or ground beef L*, a*, or b* values ($P > 0.05$). CON and MDGS had higher values for consumer overall liking and texture liking of strip steaks ($P < 0.05$). Treatment did not affect flavor liking ($P < 0.05$).

In experiment 2, shoulder clods and inside rounds from 24 forage-finished steers were ground in groups, divided into five 35 kg batches, and assigned randomly to one of five antioxidant treatments: control (CON); ground wild rice (WR); rosemary extract (ROSE); cherry seed powder (CHERRY); rosemary and pomegranate extract blend (X). Each antioxidant was added at 1% and mixed into a batch for 1 minute. Batches were formed into patties and objective and subject color scores, sensory evaluation, and TBARS were measured. L* and b* did not differ between treatment ($P = 0.49$ and 0.66, respectively), however inclusion of CHERRY did increase a* values ($P = 0.01$). Texture

liking was decreased with X compared to the WR and CHERRY ($P = 0.006$. Toughness was decreased with WR ($P = 0.03$) as compared to X and juiciness increased with the addition of CHERRY ($P = 0.003$). Overall liking, flavor liking, and off flavor were unaffected by treatment ($P = 0.09$, 0.07, and 0.06, respectively). TBARS values were lower with the addition of ROSE, CHERRY, and X on d0 than CON ($P = 0.0005$). WR was also lower on d7 than CON ($P < 0.0001$).

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Introduction

The influence of diet on livestock is an important for a number of key reasons. Not only is animal performance and growth rate impacted, but the products obtained from these animals are influenced as well. The quality of both fresh and processed meat products can be altered through changes in animal's diet. Arguably one of the most important factors in meat products that can be altered through diet is the amount, type, and quality of lipids that are deposited in the adipose tissue (Wood et al. 2008; Smith et al. 2009; Woods and Fearon, 2009). A change in composition of adipose tissue can have a cascading effect on other quality attributes such as objective and subjective color measurements (Segers et al. 2011), sensory characteristics (Aldai et al. 2010), and texture (Mello Jr. et al. 2012). Additionally, a change in lipid composition can greatly impact the rate of lipid oxidation in a meat product (Luciano et al. 2011; Mello Jr. et al. 2012) as well as the previously mentioned quality attributes.

The proceeding review of literature and research will evaluate the impact of changing dietary components on fresh and processed beef quality, with a focus on lipid composition and oxidation. Of particular interest in the diet, will be the replacement of conventional corn-soybean based diets with byproducts from the corn ethanol and soy biodiesel industries as well as changes in management of traditional feedlot animals to a pasture finished system. Lipid composition and oxidation rates as well as the subsequent

changes to color, sensory traits, and texture will be further explored in both fresh and processed meat products from animals fed these varying diets.

Grass finishing beef cattle

With growing health concerns by consumers and increasing demands for more "naturally" raised beef, there has been an increase in finishing beef animals on pasture rather than in a conventional feedlot setting. As an alternative management system, grass fed cattle may finish more slowly than conventional feed lot cattle depending on forage type and availability as well as breed. For example, Bagley and Feazel (1987) state that a mixture of cool and warm season forages such as ryegrass and clover in the fall and winter with Bermuda grass in the spring and summer, offer the best options to forage finished cattle to improve performance. Warm season grasses many be more abundant and provide a great quantity of nutrients, but cool season grasses generally have a higher quality of these nutrients (Bagley et al. 1984; Wilson, 1984). With different forages it is important to note that animal performance may differ and lead to higher or lower finishing weights, which will likely impact carcass traits as well (Dierking et al. 2010).

In addition to forage selection, breed may be of importance when considering pasture finishing cattle. Bressen et al. (2011) investigated the impact of forage finishing beef animals versus grain finishing, and also the influence of *Bos indicus* or *Bos taurus* breeds. With pasture finishing, there were no differences in the deposition of saturated fatty acids or monounsaturated fatty acids, but polyunsaturated fatty acid concentrations were decreased in *Bos taurus* cattle.

Additional information on the impact grass finishing has on carcass traits, meat quality characteristics, and lipid composition and oxidation will be explored in later sections.

Corn and soybean byproducts in beef finishing diets

As an alternative to corn in beef cattle finishing diets, byproducts from the ethanol industry have been used to alleviate rising corn costs. Many studies (Vander Pol et al. 2006; Gill et al. 2008; Depenbusch et al 2009; Leupp et al. 2009; Walter et al. 2010; Wood et al. 2011) have been conducted to evaluate the use of distillers grain products in feed lot cattle diets on performance as well as meat quality characteristics.

For example, Huls et al. (2008) concluded with modified distillers grains (MDGS), average daily gain (ADG) was highest in cattle with an inclusion of 20 to 30% MDGS, and gain to feed ratio (G:F) was the lowest at 40 to 50% MDGS inclusion. They also suggested that MDGS could be included up to 50% of the diet DM without detrimental effects. Trenkle (2007, 2008) completed two studies evaluating MDGS and found that up to 47% MDGS can be fed to finishing steers without affecting performance in the feedlot or carcass value, however inclusions as high as 60% MDGS or WDGS on a DM basis did reduce feed intake, ADG, and carcass value.

Additionally, soy byproducts from the biodiesel industry have become more available to be used in cattle finishing diets. Hales et al. (2012) found that including crude soybean glycerin in one experiment tended to decrease feed efficiency, but increased body weight and ADG in another. Additionally, Parsons et al. (2008) also

found increases in ADG with the inclusion of glycerin, as long as it remained below 8% of dietary DM. However, they also found an increases in feed efficiency with glycerin inclusions as high as 12%. In another study, (Mach et al. 2009) found no differences in ADG, G:F, or dry matter intake with the inclusion of glycerin.

Changes to live animal performance from the addition of corn and soy products may influence carcass characteristics and meat quality in beef animals. These impacts will be looked at in the following sections.

Dietary influence on carcass characteristics

One factor to consider with carcass characteristics is grass finished cattle versus conventional feedlot cattle. Feedlot cattle have heavier carcasses, larger longissimus muscle (LM) areas and more backfat at the 12^{th} rib with grass finished cattle having leaner carcasses (Kim et al. 2012). This is not surprising as feedlot cattle diets tend to be more energy dense, allowing cattle to accumulate more fat, particularly subcutaneous fat (Mandall et al. 1998; Kim et al. 2012). Kerth et al. (2007) also observed larger LM areas, more backfat, heavier carcasses, and higher USDA Yield Grades for concentrate fed steers versus ryegrass finished animals. However, marbling and subsequently USDA Quality Grade were unaffected. There is also a noted difference when comparing different forages between different grass finished animals. For example, Dierking et al. (2010) observed that when Angus steers were finished on red clover they had larger LM areas and greater finishing weights, and therefore hot carcass weights, than steers grazing alfalfa pastures.

As beef producers continue to look for alternatives and byproducts to replace corn for a more economical feeding program, researchers are continuing to evaluate the impact these dietary changes will have on carcass characteristics such as LM area and backfat thickness (Parsons et al. 2008), hot carcass weight (HCW; Parsons et al. 2009), and USDA Quality and Yield Grades (Schneider et al. 2010). Feeding low levels of a soy byproduct in the form of crude glycerin have been shown to have no effect on any carcass traits, but did however show a reduction in the percentage of carcasses that were grade as USDA Choice or higher and increased those graded USDA Select overall (Schneider et al. 2010). Additional work with crude glycerin in cattle reported varying results. In one study by Parsons et al. (2008), LM area, marbling score, and backfat thickness were decreased with feeding glycerin. Decreases in these traits likely led to the increase in USDA Select carcasses also noted in this study. Another study from the same group (Parsons et al. 2009) showed similar decreases in LM area and marbling, but did report increases with HCW with the addition of low levels of crude glycerin (less than 8%). However a study by Mach et al. (2009) resulted in no differences for any carcass traits when glycerin was added at up to 12% in animals fed high concentrate diets. Little data are available regarding the effects of adding soybean based glycerin to cattle finishing diets beyond fresh carcass traits and addressing further processed meat quality characteristics.

When considering the effects of corn byproducts on beef carcass characteristics, much of the research shows no differences for carcass traits when byproducts (such as distillers grains) are used to replace corn or other grains. Leupp et al. (2009) found that

the addition of 30% dried distillers grains plus solubles (DDGS) to either the growing or finishing diets of cattle had no effect on any of the measured carcass characteristics including LM area and backfat thickness which in turn did not affect USDA Quality and Yield grades. No changes to carcass traits such as LM area, backfat thickness, HCW, and kidney, pelvic, and heart fat percentage (KPH %) were found in several other studies that replaced corn with DDGS at various levels of inclusion (Schoonmaker et al. 2013; Segers et al. 2011; Wood et al. 2011). With these carcass traits unchanged, USDA Yield grades also were unaffected in these studies. Although DDGS contains approximately three times more protein, fat, fiber, and phosphorus than corn (Klopfenstein et al. 2008), it seems that replacing corn with these byproducts does not negatively impact carcass traits, but may have an impact on fresh and processed meat products.

Dietary influence on meat quality attributes

Beef quality attributes such as objective color, flavor, and texture must be considered when diets are altered. However replacing corn with ethanol and biodiesel byproducts has had variable results on various meat quality attributes, including objective color, flavor, and texture. Some of these variations in results have been attributed to the differences in the products themselves (wet vs. dry distillers grains; Cao et al. 2009; Luebbe et al. 2012) and the source or type of the grain for ethanol production (Gill et al. 2008; Depenbusch et al 2009; Walter et al. 2010; Wood et al. 2011). In 2002, Spiehs et al. showed a marked difference in the nutritional content of corn from the upper Midwest used for ethanol production and a resulting difference in the byproducts. Also, they noted

that nutrients such as crude fat were higher than previously reported values (NRC 1998). Cromwell et al. (1993) also found variability in the quality and nutrient value of DDGS sampled from 9 different beverage and fuel ethanol plants. These nutritional differences may lead to some of the varying results with feeding distillers grains to cattle.

Color

The majority of meat color is influenced by the heme protein myoglobin (Suman and Joseph, 2013). Within the center of the structure resides a heme ring with an iron core. The color that is seen in most fresh meat products will depend on the oxidation state of the iron molecule (ferrous or ferric) and what is attached at the sixth ligand position. Four nitrogens and the remaining protein portion of myoglobin bind the remaining five positions on iron (Young and West, 2001). When oxygen is bound to Fe^{2+}, beef is a bright, cherry red. When iron is oxidized to Fe^{3+}, oxygen is lost and water is bound. The resulting reaction is a brown discoloration. However, if meat is stored in the absence of oxygen (such as a vacuum package) and iron has not oxidized, a deep reddish purple color is the result (Schwartz et al. 2008). The three main states of myoglobin are called: deoxymyoglobin (deep reddish purple color), oxymyoglobin (bright, cherry red), metmyoglobin (brown discoloration; Suman and Joseph, 2013).

The rate of oxidation and reduction to the iron core is dependent on the environment in which the meat is stored and also the composition of the meat itself (Mancini and Hunt, 2005). One important factor is the lipid composition of the meat and its rate of oxidation (Faustman et al. 1999). Secondary lipid products from lipid

oxidation have been shown to act as prooxidants for myoglobin, thus causing an increase in the rate of brown discoloration on the surface of fresh meat products (Faustman et al. 2010). Diet change and its impact on lipid composition and oxidation will be discussed in more detail, however it is worth considering the impact diet alterations, such as the introduction of byproducts or the practice of grass finishing, can have on color stability and shelf life in meat products.

Pasture finishing cattle has been shown to have an effect on objective color scores (CIE, L*, a*, b*) in beef. L* values define the lightness of a product, a* values represent the redness, and b* the yellowness. Bidner et al. (1981) found a decrease in L* values and that beef carcass from forage finished animals were darker in appearance than those fed conventional feedlot diets. Additional studies (Abdullah et al. 1979; Couse and Seideman, 1984) also found the lean of grass finished beef animals to darker, however Sapp et al. (1998) and O'Sullivan et al. (2003) did not find any significant differences in lean color between grass and concentrate finished beef. Warren et al. (2007) observed that b* values were higher in cattle that were fed grass silage compared to those fed a high concentrate diet. Kerth et al. (2007) found similar results between pasture finished steers and concentrate steers, with the pasture finished animals having the highest b* values. Increased b* values indicate a more yellow coloring and this increase is likely due to the increase in yellow and orange pigments (carotenes) deposited in the fat of pasture finished cattle from the grasses.

While there are little data on the effects of DDGS in beef diets on objective color of beef, what is available is inconsistent for fresh beef products. Luepp et al. (2009)

found that when 30% DDGS was included in the grower, finisher, or grower and finisher diets of beef cattle, L* values decreased as did a* values when it was included in finisher diets. However, when Segers et al. (2011) added DDGS at 25% of the diet to beef cattle, a* values remained higher for six days during retail shelf life display in steaks. In yet another study by Gill et al. (2008), control groups fed steam flaked corn had lower L* values but higher a* values than treatments fed 15% corn distillers grains. Currently there are no studies evaluating the effects of crude soybean glycerin inclusions on beef color and this needs to be further explored.

Flavor

R. C. Lindsay (2008) noted that long chain polyunsaturated fatty acids (C>18,) such as arachidonic acid, found in fat in livestock can contribute a great deal to the characteristic "animal" or "meat" flavor we associate with cooked meats like beef. In fact, increases in polyunsaturated fatty acids in adipose tissue may increase oxidation, which can in turn lead to the development of off odors and flavors (Pearson et al. 1977). Changes to this delicate lipid composition from diet alterations can leave a marked impact on the flavor or sensory characteristics of these meat products.

With grass finished beef, Kerth et al. (2007) showed that flavor intensity and overall beef flavor were higher in steers finished on a high concentrate diet rather than those on 100% ryegrass pasture. Maughan et al. (2012) sought to develop a beef flavor lexicon for grass finished beef and found that beef from animals that were pasture finished scored higher in intensity for flavor attributes like "gamey", "grassy", and

"barny" as well as being more bitter compared to cattle fed conventional concentrate diets. This is not unexpected as many compounds in the forage are fat soluble and deposited within the adipose tissue. Even though they are likely leaner, the flavor will still be more intense as the distinctive flavors are attributed to beef fat, particularly linolenic acid (C18:3; Griebenow et al. 1997). This leanness is likely attributed to lower juiciness scores in the grass finished cattle as well. In both the previous studies, grain finished animals scored higher in consumer overall acceptability score. Cox et al. (2006) also found that consumers polled in three southeastern state grocery chains preferred grain finished beef, but when consumers were asked to take beef home to prepare themselves, they found no difference between grass or grain fed beef.

Considering again that DDGS contain about three times more fat than corn, replacing it in the diet may lead to flavor changes in the meat. Ruminants, however, do have the ability to alter or buffer major changes to lipid composition in the diet through biohydrogenation (Bauchart, 1993; Jenkins, 1993). This is likely why diet changes have a more pronounced effect on monogastric species and meat quality as they are unable to manipulate fatty acids in the same capacity. When feeding cattle 30% DDGS during the finishing or both the growing/finishing phase, Leupp et al. (2009) found steaks from these animals to be more juicy and flavorful. In another study, cattle fed 20% and 40% corn DDGS had higher consumer scores for tenderness and palatability, while the 20% treatment scored higher in beef flavor intensity and desirability (Aldai et al. 2010). The data indicate that the use of DDGS in finishing diets increases beef flavor intensity and possibly juiciness.

Texture

As is the case with color scores and sensory characteristics, the texture of a meat product can be impacted by diet manipulation, however not usually to the same degree. With fresh beef products, objective and subjective tenderness are often the most commonly studied attributes. One of the most common tests used for objective, instrumental tenderness is the Warner-Bratzler Shear Force (WBSF) test (AMSA, 1995). In short, during WBSF a core of cooked meat is sheared and the amount of force it takes to shear this core is measured, generally in kilograms of force. Cores are taken from cooked meat and parallel to the muscle fibers so that shear occurs perpendicular to these fibers.

With the addition of DDGS in the diet, WBSF findings are variable. After seven days of aging, for example, steaks from DDGS fed cattle were more tender (lower WBSF values) than those from conventional cattle (Segers et al. 2011). In the same study, values were similar for DDGS and conventional cattle after 14 and 21 days of aging. Leupp et al. (2009) and Gill et al. (2008) found no differences in tenderness when 30% or 15% distillers grains respectively were added to cattle diets. As for grass finished beef, Kerth et al. (2007) found that cattle grazed on only ryegrass had higher WBSF values than did their counterparts on grain diets. However, other studies (Sapp et al. 1998; Cox et al. 2006; Kim et al. 2012) showed no differences in shear force values between pasture and grain finished beef.

Overall there is no clear determination the extent in which changing diet components will impact the texture, particularly the tenderness of fresh beef products.

Diet impact on lipid composition and oxidation

While color, texture, and other sensory traits may be impacted by a dietary change, lipid composition of muscle and adipose tissue will likely be altered as well. The percentage change in individual fatty acids due to diet alterations is most noticeable in monogastric species as the fatty acids will pass through the digestive system relatively unchanged before deposition into muscle and adipose tissue (Berg, 2001). This is where we see the most dramatic changes to lipid composition and quality when by products are introduced into the diet, and several studies (Engel et al., 2001; Rentfrow et al., 2003; Stein and Shurson, 2009) have evaluated these changes and their effects on meat quality. However, unlike monogastrics, ruminants can alter feedstuffs in the rumen because of the microflora present. This ability may impact the composition, quality, and oxidation rate of fatty acids in beef products.

Lipid structure

To appreciate how changes to fatty acids in the rumen and adipose tissue can impact the quality and eventually oxidation of lipids, an understanding of their structure is needed. Fatty acids are a group of lipids characterized by a carboxyl group followed by a carbon chain or tail. Straight-chained, even numbered, long chained fatty acids (greater than 12 carbons) are the main focus of most lipid profiles in meat products.

Many times these fatty acids found in animal adipose tissue can be categorized into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA; McClements and Decker, 2008). Saturated fatty acids are those that lack any double bonds along their carbon chain. They generally have higher melting and smoke points than MUFA or PUFA and fats with high levels of these fatty acids tend to be denser or firmer in texture. The most abundant SFA is palmitic acid (C16:0), however stearic acid (C18:0) is of great interest particularly in beef products. Monounsaturated fatty acids have one double bond along their chain and the most abundant of these (and all fatty acids) is oleic acid (C18:1; Wood et al. 2003). Other isomers of C18:1 are important to note as well like trans vaccenic acid (C18:1n-11t; TVA). Vaccenic acid is an important intermediate during the process of biohydrogenation within the rumen and also a precursor to conjugated linoleic acid (CLA), which has been shown to have significant health benefits especially as an anti-carcinogen (Daley et al. 2010). Conjugated linoleic acid and other fatty acids with more than one double bond are considered PUFA. Polyunsaturated fatty acids are unable to pack as densely together because of the bends that form in the carbon chain when a double bond is present. This leads to fats high in PUFA to have an oilier and softer texture as well as lower melting points and increased rates and susceptibility to lipid oxidation (McClements and Decker, 2008). Polyunsaturated fatty acids of interest are linoleic (C18:2) and linolenic (C18:3) acids. Increases in these two fatty acids can cause potential meat quality issues such as reduced shelf life stability (Younathan and Watts, 1959; Wood et al. 2003), decreased retail display time due to discoloration (Faustman et al. 1999), and the formation of off

odors and off flavors (Pearson et al. 1977), most stemming from an increase in oxidation rate as mentioned above. Pork products tend to be higher in both linoleic and linolenic acid with 14.3% and 1.4% average compared to the 1.1% and 0.5% found in beef (Wood et al. 2008) and cause increased concern with the addition of byproducts in swine diets as they tend to raise the level of total PUFA in subcutaneous tissue when fed. However, this increase can occur in beef as well, although generally not to the same extent nor to the same degree of quality deterioration.

Additionally, fatty acids can be categorized by the configuration of the hydrogens at a double bond site. These are in either a cis or trans configurations. Most unsaturated fatty acids (UFA) are normally cis configuration, where both hydrogens are oriented on the same side of the double bond. In a trans configuration, the hydrogens are on opposite sides of the double bond (McClements and Decker, 2008).

Dietary effects on fatty acids in the rumen and lipid deposition in beef adipose tissue

Ruminant animals have the unique ability to modify lipids from feed. The microbiological flora present can add hydrogens and saturate unsaturated fatty acids through a process known as biohydrogenation. Briefly, as triacylglycerols enter the rumen, individual fatty acids are removed from their glycerol backbone by hydrolysis via lipase action. Isomerization via isomerase occurs and conjugated isomers are formed. It is with these conjugated isomers on which hydrogens are added at double bond sites, creating saturated fatty acids that will pass into the omasum (Bauchart 1993; Jenkins 1993; Jenkins et al. 2008). This process accounts for the increase in the percentage of

saturated fats present in the meat and adipose tissue of ruminant animals compared to non-ruminants (Wood et al. 2008). However, alterations to the diet by the addition of by-products or grass finishing may impact the fatty acid concentrations in the rumen fluid. Aldai et al. (2012) showed that feeding beef cattle DDG increased total SFA in the rumen fluid, particularly stearic acid, and also decreased several cis-18:1 isomers. These changes may be reflected in the lipid composition of the adipose tissue in beef products.

As a reference, Wood et al. (2008) states that averages for stearic, oleic, linoleic, and linolenic acids in subcutaneous adipose tissue for beef cattle as a percentage of all fatty acids are 12.2, 35.3, 1.1, and 0.5% respectively. However when cattle were fed DDGS, oleic acid and MUFA were decreased as compared to those found in control diets (Segers et al. 2011), but was still within the normal range at 36.3%. In the same study, total PUFA, particularly linoleic and linolenic acids, were increased in the subcutaneous fat, while stearic acid content remained unchanged. Gill et al. (2008) found different results when feeding DDG. While PUFA concentrations did increase with the addition of DDG, stearic acid also increased with the addition of ethanol byproducts along with vaccenic acid (C18:1n-11t). These differences may be partially due to the variation in distillers grains products mentioned earlier and by Spiehs et al. (2002). Differences in crude fat content may not only impact the lipid profile of the feed, but eventually the lipid composition of the adipose tissue as well. The effects of crude soybean glycerin have been unclear as to the extent in which lipid profiles may be altered.

Finishing cattle on pasture has shown consistent results in changing the lipid profile in the adipose tissue which increases PUFA concentrations and decreases total

SFA. Bressan et al. (2011) evaluated the effects of finishing system on beef cattle and found that there was a decrease in oleic acid concentration for animals finished on pasture while there was an increase in n-3 fatty acids as a percentage of total PUFA. Additionally, Fincham et al. (2009) showed that linolenic acid concentration as well as CLA concentrations were higher in pasture finished animals than feedlot cattle. Kronberg et al. (2011) evaluated if supplementing grazing cattle with flaxseed or corn and soybean meal affected fatty acid profile. Supplementation of grain during grazing resulted in higher concentrations of n-3 fatty acids and linolenic acid for steers that received flaxseed, but not the corn and soybean meal. However, total SFA, MUFA, and PUFA were unaffected. It should be noted that in these studies, grass finished animals were leaner than their grain finished counterparts.

Although diet does not change ruminant adipose tissue as drastically or in as short a time frame as it does with monogastrics, there is still a noticeable effect from diet alterations or finishing systems. While biohydrogenation does alter a great deal of lipids passing through the rumen, increases in PUFA in the feed composition may be high enough for some to pass through the rumen unchanged.

Diet and lipid oxidation

Changes to fatty acid profiles, particularly an increase in total MUFA and PUFA, can impact the rate of lipid oxidation in beef products. Fatty acids that have double bonds, as MUFA and PUFA do, are more susceptible to lipid oxidation and degradation, often producing secondary oxidation products that produce additional free radicals, off

odors, or off flavors in meat products (Pearson et al. 1977; Esterbauer et al. 1991). As the number of methylene-interrupted carbons increases, the rate of oxidation doubles, generally shortening the shelf life stability and retail display time (Wood et al. 2008). To understand how these changes may influence the resulting beef product a brief summary of lipid oxidation is needed. Lipid oxidation can be categorized into three steps: initiation, prorogation, and termination. During the initiation step, the abstraction of hydrogen from the fatty acid at a double bond site begins. A free radical, often an alkyl radical (L•), is responsible for removing this hydrogen and an alkyl radical (L•) is now present at the methylene-interrupted carbon and resonates between the two adjacent carbons (Tejero et al. 2004). For example, in linoleic acid the double bonds are most often located at carbon 9 and carbon 12. A hydrogen would be removed from carbon 11 (the methylene-interrupted carbon) and the resulting free radical would resonate between carbons 9 and 13. Often this causes conjugation of the fatty acid and a trans configuration of the remaining hydrogens is developed (Ladikos and Lougovois, 1990).

Propagation begins with the addition of O_2. Oxygen is easily available in fresh beef products as most packaging allows for the exposure of oxygen to maintain a favorable bright, cherry red color. O_2 combines with the existing free radical on the fatty acid and a peroxyl radical ($LOO^{\bullet}$) is formed. Another hydrogen is then abstracted from another free fatty acid or L•. The combination of this additional hydrogen with the $LOO^{\bullet}$ on the fatty acid forms a hydroperoxide (LOOH; Gray, 1978). During the hydrogen abstraction to complete the LOOH, another L• was likely formed on an additional fatty acid propagating oxidation of more and more fatty acids. Prooxidants can also increase

or prolong propagation. Prooxidants are compounds that can increase the rate of oxidation in a product. Common prooxidants that can increase oxidation in beef products include oxygen, light, transition metals like iron and copper, and even increases in temperature (Ingold, 1962).

For termination to take place the system can lack hydrogens needed for the formation of additional radicals, two free radicals can interact, or antioxidant compounds can interfere. The mechanics of antioxidants and neutralizing free radicals will be discussed in a later section.

The hydroperoxides (LOOH) that are formed as a result of lipid oxidation are unstable and decompose quickly (Tejero et al. 2004). During decomposition $OH^{\bullet}$ is moved from the remaining $O^{\bullet}$, which is an alkloxy radical. The hydroxyl radical ($OH^{\bullet}$) released in this process can interact with other fatty acids or methylene-interrupted carbons and begin oxidation again. The alkloxy radical remaining on the fatty acid chain has enough energy to cleave the covalent bonds in the carbon chain through a process called ß-Scission. This ß-Scission or splitting of the carbon chain results in the formation of a variety of secondary oxidation products. What products develop depend on the starting fatty acid, where the ß-Scission occurred on the carbon chain, and what other groups interact with it. ß-Scission can happen numerous times along a chain or on already formed secondary products to create shorter and shorter chained products (Mottram, 1987).

Aldehydes, ketones, alcohols, acids, and vinyl radicals are some examples of additional products that form due to ß-Scission (Esterbauer et al. 1991). Cleavage

towards the carboxyl end, for example, may result in a short chain aldehyde. A short chained aldehyde of great interest is malondialdehyde (MDA) because it's very reactive and potentially mutagenic and carcinogenic (Esterbauer et al. 1991). As mentioned these secondary oxidation products can negatively impact several meat quality traits. An example of impacting quality would be secondary oxidation products reaction with myoglobin, the protein responsible for the majority of meat color (Suman and Joseph, 2013). Increased lipid oxidation and secondary oxidation product formation compromises myoglobin stability and these effects are seen as browning in meat products (Faustman et al., 1999).

One of the most common ways to evaluate lipid oxidation is to measure the secondary oxidation products, particularly MDA. This common method measures thiobarbituric acid reactive substances (TBARS; Tarladgis et al. 1960). Knobel et al. (2013) showed that the addition of wet distillers grains (WDG) did not change TBARS values between the treatment and the control groups of cattle. However, in another study, feeding WDG increased TBARS values and therefore lipid oxidation (de Mello et al. 2007). Likely these differences are effects of whether or not the fatty acid profile was altered due to dietary differences.

As discussed previously, there is an increase in concentration of unsaturated fatty acids when cattle are pasture finished, as compared to concentrates such as corn. Luciano et al. (2011) found that finishing cattle on grass silage did in fact increase total PUFA concentrations and also increased TBARS values in beef from animals fed either pasture alone or pasture with grains supplementation compared to cattle feed a high concentrate

diet. However, Warren et al. (2007) noted an increase in lipid oxidation in beef from cattle fed a diet of high concentrates compared to grass finished animals. The authors attributed this to the increase levels of vitamin E in the plasma and muscle of the grass fed cattle, as vitamin E is a very effective antioxidant. Additionally, Gatellier et al. (2005) also showed lower lipid oxidation values in cattle that were pasture finished versus those fed a mixed diet of silage and concentrates, even after finding higher PUFA levels in the exclusively pasture finished animals. Other studies have shown similar results reporting increases in PUFA concentration in grass finished animals, which would mean increased oxidative potential, but also increased levels of vitamin E from the pasture (Warren et al. 2002; Wood et al. 2003; Campo et al. 2006).

Considerations of oxidation in processed meat products

There are some under reported areas concerning diet impact on meat quality. These include the effects of byproducts and grass finishing on the meat quality characteristics of further processed meats, particularly when changes to lipid composition and quality are impacted. As mentioned in the previous section, increasing unsaturated fatty acids can increase lipid oxidation rate as well as impact the texture of process products. While there may be some human health benefits from increasing PUFA in animal fats, they can pose several challenges to meat quality, particularly that of processed products that must withstand mechanical shearing, cooking temperatures, and increased exposure to prooxidants such as heavy metals, light, oxygen, and salts. Fats high in unsaturated fatty acids will have a softer texture and a lower melting point which

cause further processed meat products to have a soft or mushy mouth feel as well as having processing issue such as greasing out or the formation of fat caps.

Role of Antioxidants

Increased rates of oxidation from diet alterations and mechanical, chemical, and oxidative stressors from processing, highlight the need for natural and synthetic antioxidants (Wood et al. 2008). Natural antioxidants can be inherent to the meat product, such as increased vitamin E in grass finished cattle (Warren et al. 2007; Daley et al. 2010), or from added products like fruits, herbs, or other plant products (Descalzo and Sancho, 2008). Common compounds that are found in fruits and other plant products with known antioxidant capacities are vitamins E and C (α-tocopherol and ascorbic acid respectively), carnosic acid, anthocyanins, and other phenolic compounds (Karre et al. 2013). Synthetic antioxidants are also commonly used in meat products and include butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT), and tertiary butylhydroxyquinone (TBHQ; Colindres and Brewer, 2011).

Along with consumer interest in more "naturally" raised livestock, is also the interest in using natural additives in meat products, leading to antioxidants from fruits and plant products becoming more and more common to use in place of synthetic antioxidants. The use of natural compounds can help to make products and their labels more appealing to discerning consumers. However, both natural and synthetic compounds share a common aspect that makes them effective antioxidants: the phenolic ring and ability to neutralize free radicals (Karre et al. 2013).

Antioxidants, or free radical scavengers, have the ability to donate a hydrogen to a free radical, eliminating it from circulation and attacking free fatty acids. However, the antioxidant then contains a free radical. The antioxidant compound can then pass the free radical around several locations on the phenolic ring through a process of called resonance delocalization. This lowers the energy of the free radical making it much less likely to interact with a free fatty acid (McClements and Decker, 2008). Most of these compounds act during the propagation or termination stages of lipid oxidation and are more likely to react with peroxyl radicals, but also block the formation of additional alkyl radicals on free fatty acids and the formation of hydroperoxides and their eventual decomposition leading to β-Scisson (Ladikos and Lougovois, 1990).

Excellent examples of this process occur with α-tocopherol and carnosic acid. Carnosic acid is one of the main antioxidant compounds in rosemary oils and extracts and rosemary is one of the most recognized sources for antioxidants. Sebranek et al. (2005) actually found natural rosemary extracts to be more effective in reducing TBARS values and therefore lipid oxidation in pork sausage than BHT and BHA. Gibis and Weiss (2012) found grape seed to be more effective than rosemary extracts in fried beef patties. As for α-tocopherol, it is found in a variety of food stuffs, particularly green leafy vegetables. This accounts for the increase in α-tocopherol that can be associated with finishing beef cattle on pastures. Additionally, α-tocopherol can work with ascorbic acid to be more effective. Ascorbic acid can act synergistically with α-tocopherol to transfer a free radical from α-tocopherol to an ascorbic acid compound, thus regenerating α-tocopherol to continue scavenging free radicals (McClements and Decker, 2008).

Additional sources of antioxidants that have been used in beef products are found in the hulls of wild rice. Asamarai et al. (1996) and Johnson et al. (1996) found that the addition of ground wild rice to beef patties reduced lipid oxidation as seen with lower TBARS values compared with controls.

Several studies have been conducted to evaluate the effects that a variety of natural antioxidant compounds have had on the quality and lipid oxidation rate of beef products. For example, grass fed beef has been repeatedly shown to have higher levels of PUFA and therefore an increased oxidative potential. Several studies have also shown that grass fed beef is higher in vitamin E, which helps to reduce lipid oxidative in a system that maybe more susceptible (Warren et al. 2002; Wood et al. 2003; Gatellier et al. 2005; Campo et al. 2006; Warren et al. 2007).

Conclusion

Diet alterations and manipulations can have an extremely important role in the quality of beef products. A product's fatty acid profile, lipid quality, oxidation rate, and consumer appeal can all be directly impacted by changes in beef finishing diets which will in turn effect color, texture, shelf life stability, flavor, and other sensory attributes.

Two areas of dietary alteration that are growing in the livestock industry are the addition of corn and soybean by products and finishing cattle on pasture versus high concentrate feedlot diets. Both of these practices have been shown to impact meat quality, but further research is needed to evaluate the extent in which fatty acid profiles are manipulated and processed meat quality may be influenced.

Chapter II:

Effects of Modified Distillers Grains with Solubles and Crude Glycerin Inclusion in Beef Cattle Finishing Diets on Beef Quality

K. M. Compart, J. P. Jaderborg, D. M. Paulus-Compart, G. I. Crawford, A. DiCostanzo, and R. B. Cox

Steers and heifers (n = 48) were assigned randomly to one of four treatment groups and fed individually. Treatments were as follows: steam-flaked corn diet with no modified distillers grains with solubles (MDGS) or glycerin (CON); CON with 35% MDGS (MDGS); CON with 10% glycerin (GLY); and CON with 35% MDGS and 10% glycerin (MDGS/GLY). When cattle reached a mean weight of 590 kg, they were humanely harvested at a commercial abattoir. Strip loins and shoulder clods were removed from the right side of each carcass. Treatment had no effect on any specific fatty acid ($P > 0.05$), vacuum purge loss ($P = 0.75$), cooking loss ($P = 0.40$), Warner-Bratzler shear force values ($P = 0.94$), strip steak L*, a*, or b* values ($P > 0.05$) or ground beef L*, a*, or b* values ($P > 0.05$). CON and MDGS had higher values for consumer overall liking and texture liking of strip steaks ($P < 0.05$). Treatment did not affect flavor liking ($P < 0.05$).

Introduction

Increasing corn prices as well as changes in management have led feedlot producers to look for new, more economical feedstuffs for cattle finishing diets. Co-products from the ethanol industry such as distillers grains are one of the most common choices (Depenbusch et al. 2009). Distillers grains can be used in several different forms (wet, dry, or modified) depending on the feeding needs of the producer and accessibility to these co-products. However, in the production of distillers grains the starch is removed and the crude protein, fat, and fiber levels are concentrated and increased (Aldai et al., 2010). These fats are largely polyunsaturated and increasing unsaturated fatty acid percentages in beef can lead to increased lipid oxidation and decreased color stability (Wood et al., 2003). While the fatty acid profile of ruminants is largely influenced by rumen microflora, dietary changes can potentially manipulate saturation levels (Harfoot and Hazelwood, 1997). Aldai et al. (2010) found that cattle fed either corn or wheat dried distillers grains had decreased monounsaturated fatty acids (MUFA) and increased polyunsaturated fatty acids (PUFA) as compared to the control. Saturated fatty acids remained unchanged. Gill et al. (2008) found that when 15% distillers grains were fed in beef cattle finishing diets conjugated linoleic acid (CLA) increased over the control. Dried and wet distillers grains can even vary in their effect in fat deposition. Gill et al. (2008) found that dried distillers grains increased linoleic acid concentrations compared with wet distillers grains.

Further processed meat products such as bologna and summer sausage can be negatively influenced by increases in unsaturation. Unsaturation can lead to increased lipid oxidation which can cause off flavors, off odors, and decreased color stability. Also, in response to the high temperatures and mechanical manipulation of processing meats, more unsaturated fats can "grease out" or develop fat pockets due to lower melting points and decreased fat stability and density (McClements and Decker, 2008).

Another co-product, crude glycerin, has come about due to rapid expansions in the biodiesel industry and has become an affordable feedstuff (Parsons et al., 2009). Recent studies in swine nutrition have investigated the use of this co-product in response to the unsaturation issues found with feeding distillers grains. Duttlinger et al. (2012) found that the addition of glycerin increased MUFA in both jowl and backfat and oleic acid in backfat samples, thus increasing saturation. Glycerin in beef cattle diets has been shown to potentially increase dry matter intake and feed efficiency while having no effects on carcass characteristics (Parsons et al., 2009; Schneider et al., 2010). However, many studies feeding glycerin to ruminants fail to address whether this co-product can increase saturation when fed in combination with distillers grains, as has been shown in swine and poultry. Therefore the objective of this study was to evaluate the effects of modified distillers grains with solubles (MDGS) at 35% and crude soybean glycerin at 10% inclusion in beef cattle finishing diets on carcass characteristics, meat color, fatty acid profiles, and sensory attributes of fresh and processed beef.

Materials and Methods

Treatments and Experimental Design

Forty-eight crossbred steers and heifers were fed individually using a Calan gate system and assigned randomly to one of four treatments arranged in a 2 x 2 factorial design. Cattle were divided into four pens blocked by sex and treatments were evenly distributed between pens. Treatments were: traditional steam flaked corn diet (CON); CON with 35% modified distillers grains with solubles (MDGS); CON with 10% crude soybean glycerin (GLY); CON with 35% MDGS and 10% crude soybean glycerin (MDGS/GLY). Crude glycerin and MDGS inclusion was in place of steam flaked corn in MDGS, GLY, and MDGS/GLY treatments (Table 1).

Harvest and Fabrication

When cattle reached a mean weight of 590 kg, they were humanely harvested at a commercial abattoir in two groups. Kidney, pelvic, and heart (KPH) fat percentage, hot carcass weight (HCW), ribeye area, backfat at the 12^{th} rib, quality grade, marbling score, and yield grade were collected 48 hours postmortem. Strip loins (IMPS #180A) and shoulder clods (IMPS #114) were removed from the right side of each carcass, trimmed, labeled, and vacuum packaged. Cuts were transported refrigerated to the University of Minnesota Meats Laboratory.

Strip Loins

Strip loins were weighed (Accu-weigh, Model DP-6200, Yamato Corporation, USA; Made in China) in packaging and after packaging removal to calculate vacuum purge loss percentage. Seven 2.54-cm steaks were cut serially from the anterior end of each strip loin for further analysis. One steak was weighed (Mettler, Model PM 600, Mettler Instrument Co., Highstown, NJ), suspended for 24 hours at refrigerated temperature and isolated atmosphere, and then re-weighed to calculate drip loss percentage. One additional steak was weighed, cooked (standard electric kitchen oven, Fridgidaire, General Motors, USA) to an internal temperature of 71º C, tempered to room temperature, and re-weighed to calculate cooking loss percentage (AMSA, 1995). Six cores were taken from each cooked steak and evaluated for Warner-Bratzler shear force (AMSA, 1995). Cores were averaged. Two steaks were placed on trays with polyvinylchloride (PVC) overwrap (oxygen transmission rate 1400 cc/m^2) and stored at 4º C under cool white fluorescent lighting (cool white fluorescent lighting, Sylvania H968, 100w, 2, 640 LUX) for seven days (Retail case Hussmann, GF-8, AA Equipment Company, Inc. Minneapolis, MN). Objective color values (CIE, *L*, a*, and b**) were taken at six locations on each steak with a Minolta CR-310 with illuminant D65, 2.54-cm diameter aperture, and 2º standard observer (Minolta Co., Ltd Radiometric Instruments Operations, Osaka, Japan; AMSA, 1991). Subjective color scores (lean color, surface discoloration, and overall appearance) were evaluated by an eight panelist for seven days. Lean color was evaluated on a 1-8 scale with 1 = extremely brown and 8 = extremely bright, cherry red. Surface discoloration was evaluated on 1-11 scale with 1 = 91-100%

discoloration and 11 = 0% discoloration. Overall appearance was evaluated on a 1-8 scale with 1 = extremely undesirable and 8 = extremely desirable (AMSA, 1991).

The three remaining steaks from each treatment were cooked (standard electric kitchen oven, Fridgidaire, General Motors, USA) to an internal temperature of 71° C for sensory analysis conducted by the University of Minnesota Sensory Center. Panelists were untrained consumers that were over 18 years old, had no food allergies, and had consumed beef at least twice per month. Panelists were paid for their participation. The University of Minnesota's Institutional Review Board approved all recruiting and experimental procedures. Each panelist (n = 118) received two 1-cm x 1-cm x 1-cm pieces of steak served warm from each replication of each treatment with three replications per treatment (12 samples total). They were asked to evaluate steak cubes for overall liking, flavor liking, texture liking, toughness, juiciness, and off flavor (AMSA, 1995).

Shoulder Clods

Shoulder clods were cut and ground individually, twice through a 0.375-cm grinder plate (Biro Grinder, Model 346; Biro Manufacturing Company; Marble Head, OH). Two batches of ground beef from each shoulder clod were vacuum packaged (ULTRAVAC, Model 500, Koch Equipment, LLC, Kansas City, MO) and stored frozen for bologna production. Fresh, ground beef (0.5 kg) from each shoulder clod was placed on a tray with PVC overwrap and stored at 4° C under cool white fluorescent lighting for seven days. Objective color values (CIE, *L*, a*, and b**) were taken at six locations on

each ground beef package with a Minolta CR-310 with illuminant D65, 2.54-cm diameter aperture, and 2º standard observer (AMSA, 1991). Subjective color scores (lean color, surface discoloration, and overall appearance) were evaluated by an eight panelist for seven days. Lean color was evaluated on a 1-8 scale with 1 = extremely brown and 8 = extremely bright, cherry red. Surface discoloration was evaluated on 1-11 scale with 1 = 91-100% discoloration and 11 = 0% discoloration. Overall appearance was evaluated on a 1-8 scale with 1 = extremely undesirable and 8 = extremely desirable (AMSA, 1991).

Bologna

Meat from three animals from each treatment was combined into one bologna batch. Batches were made from 11.34 kg (25 lbs) of ground beef (shoulder clods) with a commercial seasoning blend (Bologna SCTP, Newly Wed Food, Chicago, IL), 1.13 kg (2.5 lbs) of ice, sodium tripolyphosphate, and cure (Heller's Modern Cure #47688, Newly Wed Food, Chicago, IL). Ground beef and ingredients were emulsified (Alipina, PB 80-890-II Gossau S G, Switzerland, Speed setting 2, 3 knife head with Alipina tangential form blades) and then stuffed (Handtmann VF-608, Albert Handtmann Maschimen Fabrik GmbH & Co., Biberach, Germany) into inedible collagen casings (Bologna 10.8 cm Walsrober Casings, Mar/Co Sales, Burnsville, MN). Bologna was cooked to an internal temperature of 65.5º C, smoked for 1 hour (Enviro-Pak, Model CVU 500E-IT, Portland, OR), cooled overnight to 4º C and then sliced. Slices were 12-cm in diameter and 4-mm thick (Globe Slicer, Model 400, Globe Slicing Machine Co, Inc., Stamford, CT). One slice from each batch was placed on a tray with PVC overwrap and stored at 4º

C under cool white fluorescent lighting for ten days with six replications of each batch. Objective color values (CIE, *L*, a*, and b**) were taken at six locations on each slice with a Minolta CR-310 with illuminant D65, 2.54-cm diameter aperture, and 2º standard observer (AMSA, 1991). Subjective color scores (lean color, surface discoloration, and overall appearance) were evaluated by an eight member trained panel for ten days, every other day. Lean color was evaluated on a 1-8 scale with 1 = extremely brown and 8 = extremely bright, cherry red. Surface discoloration was evaluated on 1-11 scale with 1 = 91-100% discoloration and 11 = 0% discoloration. Overall appearance was evaluated on a 1-8 scale with 1 = extremely undesirable and 8 = extremely desirable (AMSA, 1991).

Sensory evaluation was conducted by the University of Minnesota Sensory Center. Panelists were untrained consumers that were over 18 years old, had no food allergies, and had consumed beef at least twice per month. Panelists were paid for their participation. The University of Minnesota's Institutional Review Board approved all recruiting and experimental procedures. Bologna slices were cut into eight sections and each untrained consumer panelist (n = 87) received two pieces from each replication with three replications per treatment served at room temperature. Panelists were asked to evaluate bologna for overall liking, flavor liking, texture liking, toughness, and off flavor (AMSA, 1995).

Fatty acid profile and TBARS

A 10 gram backfat sample was collected from the posterior end of each strip loin before cutting steaks, vacuum packaged, and stored frozen until fatty acid profile

analysis. Subsets of three animals from each treatment were selected randomly for analysis. Fatty acid profiles were determined by gas chromatography (HP 6890 series, Santa Clara, CA) with a flame ionization detector (AOCS, 1998; AOCS Ce 1-62 and Ce 2-66). Samples were run in duplicate at the Agricultural Utilization Research Institute (AURI, Marshall, MN). Samples were evaluated for individual fatty acids, total saturated fatty acids (SFA), total unsaturated fatty acids (UFA), total mono-unsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), and total trans fatty acids (TFA) and iodine value was calculated from the fatty acid profile using the most current equation from the American Oil Chemist Society (AOCS, 1998) as follows:

$$Iodine\ Number = 16{:}1\ (0.95) + 18{:}1\ (0.86) + 18{:}2\ (1.732) + 18{:}3\ (2.616) + 20{:}1\ (0.785) + 22{:}1\ (0.723)$$

Samples of each ground beef batch were collected on days 0 and 7 for analysis, vacuum packaged, and stored frozen immediately for thiobarbituric acid reactive substances (TBARS) analysis (AOCS, 1998). Secondary lipid oxidation products of lipid oxidation were measured using the thiobarbituric acid assay (Tarladgis et al. 1960). Subsets of three animals from each treatment were selected randomly for analysis at the AURI (Marshall, MN). Samples were run in duplicate and measured with a spectrophotometer (Spectronic 20^{+}, Spectronic Instruments, Inc.) at 532 nm and reported at TBARS.

Statistical Analysis

Data were analyzed as a 2 x 2 factorial design with MDGS and glycerin inclusion as main effects. Categorical data (yield grade, quality grade, and marbling score) were subjected to the GENMOD procedure of SAS (SAS Inst, Inc., Cary, NC. Version 9.1), while all other remaining data were analyzed using the MIXED procedure. Animal was considered the experimental unit and an alpha level of 5% was used to determine statistical significance. Carcasses determined to be "dark cutting" were removed from color and drip loss data, as well as not being used for bologna production. Hot carcass weight was used as a covariate for ribeye area, backfat, KPH %, yield grade, quality grade, and marbling score. Interaction between MDGS and GLY was tested, but no significance was found ($P > 0.05$) and is not shown.

Results and Discussion

Carcass Data, Moisture Loss, and Shear Force

The addition of MDGS and crude glycerin did not affect carcass measurements ($P > 0.05$) except KPH fat percentage (Table 2-2). The addition of MDGS increased KPH fat percentage ($P = 0.04$). Similarly, Schneider et al. (2010) found no differences with the addition of glycerin on most carcass characteristics with the exception of decreased percentage of carcasses grading USDA Choice. However, Parsons et al. (2009) found that the addition of more than 8% glycerin decreased hot carcass weights, Longissimus muscle area, 12th rib backfat, and marbling scores. Also, Luebbe et al. (2012) found feeding wet distillers grains (WDG) negatively affected carcass characteristics including

hot carcass weight, 12th rib backfat, and marbling scores. Similar to our results, Depenbusch et al. (2009) found no differences in carcass characteristics when feeding either wet or dried distillers grains. Glycerin and MDGS did not affect vacuum purge loss (P = 0.67 and 0.19, respectively), drip loss (P = 0.10 and 0.25, respectively), or cooking loss (P = 0.15 and 0.29, respectively; Table 2-3). The addition of glycerin did not affect Warner-Bratzler shear force values (P = 0.16), however when MDGS was added, shear force values were decreased (P = 0.03; Table 2-3).

Objective Color Scores

The addition of MDGS did not affect L*, a*, or b* in strip steaks (P = 0.90, 0.72, and 0.60, respectively). Similarly, glycerin did not affect objective color of strip steaks (L*, a*, b*; P = 0.57, 0.53, and 0.59, respectively; Table 2-4). When considering ground beef objective color, MDGS and glycerin did not affect L*, a*, or b* values (MDGS P = 0.29, and 0.98, respectively; Glycerin P = 0.25, and 0.23, respectively; Table 2-4). In the more processed product, bologna, MDGS decreased L* while glycerin increased L* values (P = 0.02 and P < 0.001, respectively). With MDGS and glycerin, a* (P = 0.78 and 0.07, respectively) and b* (P = 0.38 and 0.94, respectively; Table 2-4) were not affected. Leupp et al. (2009) found reductions in L* and b* in fresh strip steaks when cattle were fed distillers grains in the growing period and reductions in a* when fed distillers grains in the finishing period. Perhaps the addition of glycerin helped to mitigate these effects in our experiment, however no interaction was detected (P > 0.05).

Sensory, Fatty Acid Profile, and TBARS

The addition of MDGS did not affect overall liking, flavor liking, texture liking, toughness, or juiciness in cooked strip steaks ($P > 0.05$; Table 2-5). However, the addition of MDGS resulted in an increase in off flavors ($P = 0.03$). Leupp et al. (2009) found that with the addition of distillers grains in beef finishing diets, steaks were more juicy and flavorful, while tenderness remained unaffected. Gill et al. (2008) found no sensory differences when feeding wet or dry distillers grains to beef cattle. In our study, when glycerin was added, overall liking, flavor liking, and texture liking decreased ($P = 0.0001$, 0.01, and < 0.0001, respectively), while juiciness and off flavor increased ($P < 0.0001$) in strip steak samples. Toughness was not affected by glycerin ($P = 0.42$). In bologna samples the addition of MDGS did not affect overall liking or texture liking ($P = 0.06$ and 0.85, respectively; Table 2-66). However, flavor liking decreased ($P = 0.005$) while toughness and off flavor increased ($P = 0.03$ and < 0.0001, respectively).

In bologna, the inclusion of glycerin increased overall liking, flavor liking and texture liking ($P < 0.0001$) while decreasing toughness ($P < 0.0001$). Off flavor was not affected by glycerin ($P = 0.09$). The addition of co-products had no effect on any specific fatty acid ($P > 0.05$; Table 2-7). Additionally, there were no differences with MDGS or glycerin for SFA ($P = 0.35$ and 0.77, respectively), MUFA ($P = 0.50$ and 0.83, respectively), or PUFA ($P = 0.27$ and 0.61, respectively). However, there was an increase in total TFA with the inclusion of glycerin ($P = 0.02$). Aldai et al. (2010) found that including corn or wheat distillers grains at 20% of the diet of beef cattle did not change SFA, but decreased MUFA, specifically C18:1. They also found an increase in

PUFA when feeding 20% distillers grains. Gill et al. (2008) reported increases in C18:2 when dry distillers grains were fed at 15% of the diet. No differences were shown with the inclusion of MDGS or glycerin for TBARS values on d 0 (P = 0.63 and 0.62, respectively; Table 2-8). However on day 7 the addition of MDGS increased TBARS values (P = 0.02).

Conclusions

Results from this study suggest that the inclusion of modified distillers grains plus solubles and crude glycerin in beef cattle finishing diets did not negatively impact carcass and meat quality characteristics. Results also indicate that the addition of modified distillers grains plus solubles and crude glycerin in beef finishing diets did not negatively affect color stability of strip steaks and ground beef, but may impact sensory characteristics of beef strip steaks and bologna.

Tables

Table 2-1. Composition of dietary treatments (% of diet DM) fed in beef cattle finishing diets

	Treatment			
	CON	GLY	MDGS	MDGS/GLY
Grass hay	10.00	10.00	10.00	10.00
Steam flaked corn	81.50	70.50	51.50	41.50
Protein vitamin mineral	3.50	3.50	3.50	3.50
Protein supplement	5.00	6.00	0.00	0.00
Modified distillers grains plus solubles	0.00	0.00	40.00	40.00
Glycerin	0.00	10.00	0.00	10.00

Table 2-2. Effects of feeding crude glycerin and modified distillers grains plus solubles (MDGS) on beef carcass data

	Treatment						
	MDGS		Glycerin			P-Value	
	N	Y	N	Y	SEM	MDGS	Glycerin
Hot carcass weight, kg	371.29	369.26	367.66	372.89	13.68	0.82	0.55
Longissimus muscle area, sq cm	82.77	82.13	82.39	82.58	0.24	0.75	0.93
Kidney, pelvic and heart fat, %	2.43	2.67	2.60	2.50	0.08	0.04	0.39
12th rib backfat, cm	1.40	1.45	1.42	1.40	0.03	0.70	0.85
Yield grade[1]	2.66	2.83	2.83	2.78	2.70	0.63	0.81
Quality grade[2]	2.83	3.04	3.04	2.87	2.90	0.43	0.35
Marbling score[3]	526.86	534.58	534.58	525.61	12.15	0.62	0.20

There were no significant interactions between MDGS and glycerin

[1] Yield grade: 1 to 5 with 1= highest yielding carcass and 5= lowest yielding carcass

[2] Quality grade: 1= St, 2= Se, 3= Ch-, 4= Ch

[3] Marbling scores: 400= Slight, 500= small, 600= modest

Table 2-3. Effects of feeding crude glycerin and modified distillers grains plus solubles (MDGS) in beef cattle diets on moisture loss and shear force in strip steaks

	Treatment						
	MDGS		Glycerin			P-Value	
	N	Y	N	Y	SEM	MDGS	Glycerin
Purge loss, %	1.35	1.42	1.51	1.26	0.13	0.67	0.19
Drip loss, %	1.18	1.58	1.52	1.24	0.16	0.10	0.25
Cook loss, %	26.31	22.67	23.15	25.80	1.73	0.15	0.29
Shear force, kg	3.53	2.55	2.73	3.34	0.32	0.03	0.16

There were no significant interactions between MDGS and glycerin

Table 2-4. Effects of feeding crude glycerin and modified distillers grains plus solubles (MDGS) on objective color in strip steaks, ground beef, and bologna

	Treatment						
	MDGS		Glycerin			P-Value	
Strip steaks	N	Y	N	Y	SEM	MDGS	Glycerin
L*	37.99	37.87	37.67	38.19	0.65	0.90	0.57
a*	21.86	21.61	21.91	21.53	0.42	0.72	0.53
b*	12.25	12.12	12.11	12.25	0.17	0.60	0.59
Ground beef							
L*	36.95	36.84	37.02	36.45	0.64	0.89	0.61
a*	22.38	21.96	22.40	21.94	0.28	0.29	0.25
b*	15.58	15.58	15.72	15.44	0.16	0.98	0.23
Bologna							
L*	55.77	55.13	54.97	55.93	0.19	0.02	<0.001
a*	9.67	9.78	10.06	9.39	0.26	0.78	0.07
b*	11.55	11.35	11.44	11.46	0.16	0.38	0.94

There were no significant interactions between MDGS and glycerin

Table 2-5. Effects of feeding crude glycerin and modified distillers grains plus solubles (MDGS) on sensory attributes of cooked strip steaks

	Treatment						
	MDGS		Glycerin			P-Value	
	N	Y	N	Y	SEM	MDGS	Glycerin
Overall liking[1]	67.49	67.60	69.56	65.54	0.92	0.92	0.0001
Flavor liking[1]	69.19	68.95	70.30	67.84	0.87	0.82	0.01
Texture liking[1]	64.91	64.80	67.63	62.08	1.01	0.93	<0.0001
Toughness[2]	4.09	4.12	4.04	4.17	0.20	0.87	0.42
Juiciness[2]	9.35	8.18	6.93	7.21	0.22	0.06	<0.0001
Off flavor[2]	7.92	8.47	7.20	9.19	0.22	0.03	<0.0001

There were no significant interactions between MDGS and glycerin

[1]Liking ratings were made on a 120 point labeled affective magnitude scales, with the left most end labeled *strongest dislike imaginable* and the right most end labeled *strongest like imaginable*.

[2]Intensity ratings were made on a 20 point line scale with the left most ends labeled *none* and the right most ends labeled *extremely tough, extremely juicy,* and *extremely intense*.

Table 2-6. Effects of feeding crude glycerin and modified distillers grains plus solubles (MDGS) on sensory attributes of bologna

	Treatment						
	MDGS		Glycerin			P-Value	
	N	Y	N	Y	SEM	MDGS	Glycerin
Overall liking[1]	77.12	75.57	73.99	78.70	0.89	0.06	<0.0001
Flavor liking[1]	77.41	74.84	43.46	78.79	0.93	0.005	<0.0001
Texture liking[1]	74.80	74.65	72.77	76.67	0.96	0.85	<0.0001
Toughness[2]	3.45	3.76	3.89	3.32	0.22	0.03	<0.0001
Off flavor[2]	3.05	3.65	3.48	3.22	0.24	<0.0001	0.09

There were no significant interactions between MDGS and glycerin

[1]Liking ratings were made on a 120 point labeled affective magnitude scales, with the left most end labeled *strongest dislike imaginable* and the right most end labeled *strongest like imaginable*.

[2]Intensity ratings were made on a 20 point line scale with the left most ends labeled *none* and the right most ends labeled *extremely tough, extremely juicy,* and *extremely intense*.

Table 2-7. Effects of feeding crude glycerin and modified distillers grains plus solubles (MDGS) on beef fatty acid composition (%)

	Treatment						
	MDGS		Glycerin			P-Value	
	N	Y	N	Y	SEM	MDGS	Glycerin
Myristic, C14:0	2.93	2.82	2.97	2.77	0.20	0.71	0.50
Palmitic, C16:0	23.53	23.42	23.58	23.38	0.54	0.89	0.80
Steric, C18:0	15.31	14.87	14.82	15.36	1.14	0.79	0.75
Oleic, C18:1	44.27	45.43	44.83	44.86	0.67	0.23	0.97
Linoleic, C18:2	2.99	3.27	3.08	3.18	0.21	0.37	0.76
Iodine value	46.49	48.04	47.17	47.36	0.70	0.15	0.85
Trans fatty acids	0.38	0.40	0.31^{a}	0.47^{b}	0.04	0.72	0.02
Saturated fatty acids	44.42	43.02	43.93	43.51	0.99	0.35	0.77
Unsaturated fatty acids	52.88	54.11	53.56	53.43	0.87	0.35	0.92
Monounsaturated fatty acids	49.67	50.55	50.25	49.97	0.89	0.50	0.83
Polyunsaturated fatty acids	3.22	3.56	3.31	3.47	0.20	0.27	0.61

There were no significant interactions between MDGS and glycerin

Table 2-8. Effects of feeding crude glycerin and modified distillers grains plus solubles (MDGS) on thiobarbituric acid reactive substances (TBARS; MDA Equivalents)

	Treatment						
	MDGS		Glycerin			P-Value	
	N	Y	N	Y	SEM	MDGS	Glycerin
Day 0, mg/kg	0.44	0.47	0.41	0.44	0.05	0.63	0.62
Day 7, mg/kg	3.45	4.20	3.96	3.96	0.18	0.02	0.32

There were no significant interactions between MDGS and glycerin

Chapter III:

Evaluation of Retail Shelf Stability and Sensory Attributes of Beef Enhanced with Natural Antioxidants from Forage-Finished Cattle

K. M. Compart and R. B. Cox

Shoulder clods and inside rounds from 24 forage-finished steers were ground in groups, divided into five 35 kg batches, and assigned randomly to one of five antioxidant treatments: control (CON); ground wild rice (WR); rosemary extract (ROSE); cherry seed powder (CHERRY); rosemary and pomegranate extract blend (X). Each antioxidant was added at 1% and mixed into a batch for 1 minute. Batches were formed into patties and objective and subjective color scores, sensory evaluation, and TBARS were measured. L* and b* did not differ among treatment (P = 0.49 and 0.66, respectively), however inclusion of CHERRY did increase a* values (P = 0.01). Texture liking was decreased with X compared to the WR and CHERRY (P = 0.006). Toughness was decreased with WR (P = 0.03) as compared to X, and juiciness increased with the addition of CHERRY (P = 0.003). Overall liking, flavor liking, and off flavor were unaffected by treatment (P = 0.09, 0.07, and 0.06, respectively). TBARS values were lower with the addition of ROSE, CHERRY, and X on d0 than CON (P = 0.0005). WR was also lower on d7 than CON (P < 0.0001).

Introduction

Consumer trends and demands have led to an increase in producing and consuming forage-finished beef in the United States. While this product is usually leaner, the fat present has a higher percentage of unsaturated fatty acids which may increase lipid oxidation leading to undesirable flavors and odors (Wood et al., 2003; Fincham et al., 2009; Daley et al., 2010). Lipid oxidation is a major cause of quality deterioration in meat products, particularly in products that may be higher in unsaturated fatty acids such as forage-finished beef. The addition of antioxidants to this meat product may help to improve color and lipid stability, extending the shelf life of fresh and even processed forage-finished products. However, as consumers become more skeptical of synthetic antioxidants, some companies have begun exploring the use of natural antioxidants to make labeling more appealing to consumers and to pursue a growing interest area. Fruits, vegetables, and even hulls from some grains have been shown to have some antioxidant capacity mainly because of their high content of phenolic compounds (Asamarai et al., 1996; Cam, Hisil, and Durmaz, 2009; Karre, Lopez, and Getty, 2013). Plants that contain high levels of carnosine and anthocyanins are of particular interest, as these compounds have been shown to have a substantial antioxidant capacity, reducing lipid oxidation and increasing shelf life stability in meat products. Thus, the objective of this study was to evaluate the use of four natural antioxidants in ground beef from forage-finished cattle, evaluating color stability, lipid oxidation, and sensory characteristics.

Materials and Methods

Animals, Harvest, and Fabrication

Twenty four Angus steers were grazed on rye grass pasture at the North Central Research and Outreach Center (NCROC) in Grand Rapids, MN for approximately 450 days after an initial weaning weight averaging 225 kg. Steers were weighed every 28 days. After grazing was complete, cattle were transported to the University of Minnesota Meats Laboratory in Saint Paul, MN. Cattle were harvested in three harvest groups (8 hd/group) and final live weights, hot carcass weight, and dressing percentage were collected. Loin eye area, marbling score, 12^{th} rib backfat, cold carcass weight, and kidney, pelvic, and heart fat percentage were collected 48 hours postmortem. During fabrication strip loins (IMPS #108A), inside rounds (IMPS #168), and should clods (IMPS #114) were removed from the right side of each carcass and weighed for fabrication percentage. From the anterior end of each strip loin, seven 2.5 cm steaks were cut for further analysis. One steak was weighed (Mettler, Model PM 600, Mettler Instrument Co. Highstown, NJ), suspended for 24 hours at refrigerated temperature and isolated atmosphere, and then re-weighed to calculate drip loss percentage. One additional steak was weighed, cooked (standard electric kitchen oven, Fridgidaire, General Motors, USA) to an internal temperature of 71º C, tempered to room temperature, and re-weighed to calculate cooking loss percentage (AMSA, 1995). Six cores were taken from each cooked steak and evaluated for Warner-Bratzler shear force (AMSA, 1995). Cores were averaged. Four steaks were placed on two trays with polyvinylchloride (PVC) overwrap (oxygen transmission rate 1400 cc/m^2) and stored at

4º C under cool white fluorescent lighting (cool white fluorescent lighting, Sylvania H968, 100w, 2, 640 LUX) for seven days (Retail case Hussmann, GF-8, AA Equipment Company, Inc. Minneapolis, MN). Objective color values (CIE, *L*, a*, and b**) were taken at six locations on each steak with a HunterLab MiniScan XE Plus with illuminant D65, 2.54-cm diameter aperture, and 2º standard observer (Hunter Associates Laboratory, INC., Reston, VA; AMSA, 1991). Subjective color scores (lean color, surface discoloration, and overall appearance) were evaluated by an eight panelist for seven days. Lean color was evaluated on a 1-8 scale with 1 = extremely brown and 8 = extremely bright, cherry red. Surface discoloration was evaluated on 1-11 scale with 1 = 91-100% discoloration and 11 = 0% discoloration. Overall appearance was evaluated on a 1-8 scale with 1 = extremely undesirable and 8 = extremely desirable (AMSA, 1991). Shoulder clods and inside rounds were vacuum packaged (ULTRAVAC, Model 500, Koch Equipment, LLC, Kansas City, MO) and frozen for grinding and further analysis.

Ground Beef and Natural Antioxidants Treatments

Shoulder clods and inside rounds were ground in groups (4 animals/group; 6 groups total; 6 replications) twice through a 0.375-cm grinder plate (Biro Grinder, Model 346; Biro Manufacturing Company; Marble Head, OH). Each group was divided into five 35 kg batches (30 batches total) and assigned randomly to one of five antioxidant treatments: control (CON); ground wild rice (WR); rosemary extract (ROSE); cherry seed powder (CHERRY); rosemary and pomegranate extract (X). Rosemary, cherry seed powder, and pomegranate extract were chosen for their high levels of carnosine or

anthocyanins, while wild rice was explored based on previous research at the University of Minnesota (Asamarai et al. 1996; Johnson, Addis, and Epley, 1996; Rojas and Brewer, 2008; Cam, Hisil, and Durmaz, 2009). Each antioxidant solution was added at 1% into 1.05 kg of water then mixed (Leland Food Mixing Machine, Model 100DA; Leland Detroit Manufacturing Company; Detroit, Michigan) into its respective batch for 1 minute. Water was also added to the control treatment and mixed. Antioxidant solutions excluding the ground wild rice were obtained from Naturex (South Hackensack, NJ). Wild rice was ground with a hammer mill (Howell Electric Motors, Co.; Howell, MI) using a 0.07 cm screen into a fine powder.

Two trays of fresh, ground beef (0.5 kg) from each batch were packaged with PVC overwrap and stored at 4ºC under cool white fluorescent lighting for seven days. Objective color values (CIE, *L*, a*, and b**) were taken at three locations on each ground beef package with a HunterLab MiniScan XE Plus with illuminant D65, 2.54-cm diameter aperture, and 2º standard observer (Hunter Associates Laboratory, INC., Reston, VA; AMSA, 1991). Subjective color scores (lean color, surface discoloration, and overall appearance) were evaluated by an eight member trained panel for seven days. Lean color was evaluated on a 1-8 scale with 1 = extremely brown and 8 = extremely bright, cherry red. Surface discoloration was evaluated on 1-11 scale with 1 = 91-100% discoloration and 11 = 0% discoloration. Overall appearance was evaluated on a 1-8 scale with 1 = extremely undesirable and 8 = extremely desirable (AMSA, 1991). Remaining ground beef from each batch was immediately formed into 0.09 kg patties. Patties were frozen and stored until further sensory analysis.

Thiobarbituric Acid Reactive Substances and Sensory Analysis

Patties from each ground beef batch were collected on days 0 and 7 for analysis, vacuum packaged, and stored frozen immediately for thiobarbituric acid reactive substances (TBARS) analysis (AOCS, 1998). Secondary lipid oxidation products of lipid oxidation were measured using the thiobarbituric acid assay (Tarladgis et al. 1960). Samples were run in duplicate and measured with a spectrophotometer (Spectronic 20^{+}, Spectronic Instruments, Inc.) at 532 nm and reported as TBARS.

Sensory evaluation was conducted by the University of Minnesota Sensory Center. Panelists were untrained consumers that were over 18 years old, had no food allergies, and had consumed beef at least twice per month. Panelists were paid for their participation. The University of Minnesota's Institutional Review Board approved all recruiting and experimental procedures. Ground beef patties were cooked in an electric oven to an internal temperature of 71° C then cut into eight sections. Each consumer panelist received one piece from each treatment with two replications per treatment served warm. Panelists (n=82) were asked to evaluate ground beef for overall liking, flavor liking, texture liking, toughness, juiciness, and off flavor (AMSA, 1995).

Statistical Analysis

Data were analyzed as a randomized block design. Data were subjected to the MIXED procedure of SAS (SAS Inst, Inc., Cary, NC. Version 9.2). Animal was considered the experimental unit for carcass and strip steak data, while group was

considered the experimental unit for ground beef, sensory, and TBARS analysis. For sensory analysis, judge was considered a random effect. An alpha level of 5% was used to determine statistical significance.

Results and Discussion

Forage-Finished Steers

Tables 3-1, 3-2, and 3-3 show the carcass data, fabrication data, cook loss, shear force, objective and subjective color, and fatty acid compositions from the foraged-finished steers used for this experiment. Means are given for each harvest group and for all animals total for reference.

Objective and Subjective Color in Ground Beef

The inclusion of antioxidant treatments did not affect L* or b* (P = 0.49 and 0.66 respectively), but did have a positive impact on a* with the addition of CHERRY (P = 0.01; Table 4). Across the entire shelf life period, a* values remained higher than all treatments and CON for patties treated with CHERRY. Carpenter et al. (2007) found similar results when using grape seed extracts. L* and b* values were not impacted, but minor increases in a* value did occur with the addition of seed extracts. This increase in a* is likely due to the natural coloring of the extract as well as the relatively high concentration of phenolic compounds in fruit seed extracts. Gibis and Weiss (2012) reported that phenolic compounds in seed extracts to be quite high, even more so than rosemary extract and most synthetic antioxidants.

Additionally, lean color increased with both ROSE and CHERRY inclusions ($P < 0.0001$) compared to CON, WR and X, while all treatments aside from WR had more surface discoloration than CON ($P < 0.0001$). Overall appearance scores increased with the addition of ROSE and CHERRY over CON ($P < 0.0001$).

Thiobarbituric Acid Reactive Substances

With the addition of ROSE, CHERRY, and X, TBARS values were significantly lower on day 0 ($P = 0.0005$) and day 7 ($P < 0.0001$) than the control (Table 5). On day 7, the inclusion of WR, ROSE, CHERRY, and X also reduced TBARS values significantly compared to the control ($P < .0001$). Overall X, followed closely by ROSE, retarded secondary lipid oxidation product formation the most, increasing only from 0.04 mg/kg MDA to 0.05/mg/kg MDA for X and 0.06 mg/kg MDA to 0.07 mg/kg MDA for ROSE. The addition of seed extracts has been previously reported (Carpenter et al. 2007) to have a substantial impact on lipid stability. The addition of grape seed extract significantly lowered TBARS values over 12 days of storage as compared to controls. Rosemary extracts have long been studied and found to have substantial impacts on reducing lipid oxidation. Sebranek et al. (2005) found that the inclusion of rosemary extracts at varying levels always had lower TBARS values from the control across the entire 112 days of storage. Furthermore, in the same study it was found that rosemary extracts kept TBARS values lower than ground pork patties treated with BHT/BHA. The combination of the phenolic compounds in the fruit extract (pomegranate) and the carosine in rosemary as

the second half of the blend for the X treatment, most probably contributed to it having the greatest effect in lowering overall TBARS values in this study.

Sensory Analysis

While overall liking and flavor liking were not significantly impacted (P = 0.09 and 0.07 respectively, Table 6), the addition of X decreased texture liking (P = 0.006) compared to WR and CHERRY and increase toughness (P = 0.03) over WR. However, adding CHERRY increased juiciness compared to CON, ROSE, and X. Off flavor was not affected (P = 0.06). Both Carpenter et al. (2007) and Sebranek et al. (2005) found little to no differences in any sensory traits with the addition of either seed extracts or rosemary extracts in previous studies. It should be noted that some differences that were lower scoring, may be due to the patties being from forage-finished animals rather than a treatment effect. Only forage-finished animals were used in this study so there is no comparison with conventional grain-finished animals, which consumers may be more accustomed to.

Conclusions

In conclusion, the addition of natural antioxidant compounds to products susceptible to lipid oxidation can improve and prolong shelf life stability as shown with the inclusion of cherry seed powder which led to an increase in a* value, increased shelf stability, and an increase in juiciness for ground, pattied beef. Natural antioxidant

compounds added to ground meats more susceptible to lipid oxidation, such as forage-finished beef, may help to increase shelf life and improve overall quality.

Tables

Table 3-1. Live weight and carcass data for forage-finished steers

	Harvest Group 1	Harvest Group 2	Harvest Group 3	All Groups
Final live weight, kg	380.45	383.24	400.68	388.13
Hot carcass weight, kg	200.06	199.26	212.39	203.90
Dressing percentage, %	52.55	51.77	53.01	52.44
Longissimus muscle area, sq cm	50.90	50.65	51.23	50.90
12th rib backfat, cm	0.25	0.25	0.25	0.10
Kidney, pelvic, and heart fat, %	1.50	1.50	1.50	1.50
Cold carcass weight, kg	201.19	181.82	206.31	196.44
Marbling score[1]	420.00	420.00	420.00	420.00

[1]Marbling scores: 400= Slight, 500= small, 600= modest

Table 3-2. Fabrications percentage, moisture loss, and shear force for strips steaks from forage-finished steers

	Harvest Group 1	Harvest Group 2	Harvest Group 3	All Groups
Strip loin fabrication, %	1.60	1.56	1.48	1.55
Shoulder clod fabrication, %	2.59	2.86	2.73	2.73
Inside round fabrication, %	2.02	2.64	2.30	2.32
Drip loss, %	0.46	0.53	0.68	0.56
Cook loss, %	15.79	21.30	19.54	18.77
Warner Bratzler shear force, kg	5.05	6.58	6.78	6.14

Table 3-3. Objective color (L*, a*, b*) scores and subjective color scores for strip steaks from forage-finished steers

	Harvest Group 1	Harvest Group 2	Harvest Group 3	All Groups
L*	44.09	43.86	44.62	44.19
a*	13.20	12.38	12.45	12.68
b*	8.55	7.76	8.76	8.36
Lean color[1]	5.24	4.88	5.15	5.09
Surface discoloration[2]	8.51	4.27	7.90	7.89
Overall apperance[3]	5.06	4.54	5.00	4.87

[1]Lean color - 1 = extremely brown and 8 = extremely bright, cherry red.
[2]Surface discoloration - 1 = 91-100% discoloration and 11 = 0% discoloration
[3]Overall appearance - 1 = extremely undesirable and 8 = extremely desirable

Table 3-4. Effects of natural antioxidants on objective color (L*, a*, b*) scores and subjective color scores in ground beef patties from forage-finished cattle

	Treatment[1]						
	CON	WR	ROSE	CHERRY	X	SE	P-Value
L*	41.01	40.45	41.27	41.06	40.36	0.43	0.49
a*	7.53[a]	7.47[a]	7.82[a]	8.74[b]	7.53[a]	0.28	0.01
b*	14.91	14.92	15.37	15.88	14.90	0.55	0.66
Lean color[2]	4.34[a]	4.36[a]	4.68[b]	5.12[c]	4.39[a]	0.08	<.0001
Surface discoloration[3]	6.26[a]	6.35[ab]	6.61[b]	7.18[c]	6.61[b]	0.11	<.0001
Overall apperance[4]	4.35[a]	4.39[ab]	4.56[b]	4.88[c]	4.47[ab]	0.07	<.0001

Means within a row with different letters differ significantly

[1]Treatment – CON = control, WR= wild rice, ROSE = rosemary extract, CHERRY = cherry seed powder, X = rosemary and pomegranate extract

[2]Lean color - 1 = extremely brown and 8 = extremely bright, cherry red.

[3]Surface discoloration - 1 = 91-100% discoloration and 11 = 0% discoloration.

[4]Overall appearance - 1 = extremely undesirable and 8 = extremely desirable

Table 3-5. Effects of natural antioxidants on thiobarbituric acid reactive substances (TBARS; MDA Equivalents) in ground beef patties from forage-finished cattle

	Treatment[1]						
	CON	WR	ROSE	CHERRY	X	SE	P-Value
Day 0	0.10[a]	0.08[a]	0.06[b]	0.04[b]	0.04[b]	0.01	0.0005
Day 7	0.41[a]	0.23[b]	0.07[c]	0.18[b]	0.05[c]	0.04	<0.0001

Means within a row with different letters differ significantly

[1]Treatment – CON = control, WR= wild rice, ROSE = rosemary extract, CHERRY = cherry seed powder, X = rosemary and pomegranate extract

Table 3-6. Effects of natural antioxidants on sensory attributes in cooked ground beef patties from forage-finished cattle

	Treatment[1]					
	CON	WR	ROSE	CHERRY	X	P-Value
Overall liking[2]	61.20	62.90	62.20	64.50	61.00	0.09
Flavor liking[2]	61.40	61.70	61.70	65.60	62.70	0.07
Texture liking[2]	60.80[ab]	63.40[a]	60.60[ab]	63.90[a]	59.20[b]	0.006
Toughness[3]	5.70[ab]	5.10[a]	5.60[ab]	5.30[ab]	5.90[b]	0.03
Juiciness[3]	3.80[a]	4.20[ab]	3.90[a]	4.70[b]	3.70[a]	0.003
Off flavor[3]	3.90	4.20	4.40	3.80	4.80	0.06

Means within a row with different letters differ significantly

[1]Treatment – CON = control, WR= wild rice, ROSE = rosemary extract, CHERRY = cherry seed powder, X = rosemary and pomegranate extract

[2]Liking ratings were made on a 120 point labeled affective magnitude scales, with the left most end labeled *strongest dislike imaginable* and the right most end labeled *strongest like imaginable*.

[3]Intensity ratings were made on a 20 point line scale with the left most ends labeled *none* and the right most ends labeled *extremely tough, extremely juicy,* and *extremely intense*.

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Appendix A

Fatty Acid Composition Analysis

I. **Reagents / Materials**

A. GLC 60 Reference Standard: Nu-Chek cat. # GLC 60, 4 x 25 mg ampoules

B. GLC 67 Reference Standard: Nu-Chek cat. # GLC 67, 4 x 25 mg ampoules

C. GLC 80 Reference Standard: Nu-Chek cat. # GLC 80, 4 x 25 mg ampoules

D. GLC 90 Reference Standard: Nu-Chek cat. # GLC 90, 4 x 25 mg ampoules

E. Boron Trifluoride Methanol Solution, 14%

F. Sodium Hydroxide

G. Methanol

H. Sodium Chloride

I. Sodium Sulfate, Anhydrous, Granular

J. Heptane

K. 0.5N Methanolic Sodium Hydroxide: Dissolve 20 g of sodium hydroxide in 1000 mL of methanol. Mix well.

L. Saturated Sodium Chloride Solution: Add sodium chloride to 1000 mL of deionized water until no more sodium chloride visually dissolves. Mix well and filter solution.

M. Borosilicate Glass Beads

N. Compressed Air

O. Helium

P. Hydrogen

Q. Autosampler Vials: 2 mL wide top crimp

R. Autosampler Vial Crimp Caps: 11 mm silver aluminum, clear PTFE/red natural rubber septa

II. **Instrumentation**

A. Gas Chromatograph: Hewlett-Packard 6890 Plus Gas Chromatograph equipped with a split/splitless injection port and autosampler.

B. Detector: Hewlett-Packard Flame Ionization Detector.

C. Data Acquisition System: Hewlett-Packard ChemStation chromatography software.

D. Analytical Column: SP™-2560, 0.25 mm ID x 100 m, Supelco

E. Injector Liner: split, straight, glasswool, non-deactivated

F. Non-Stick Flip-Top Liner O-Ring

G. Inlet Septa: general purpose red septa

H. Column Ferrule: Graphite (short) Ferrule, 0.5 mm ID

I. Gas Purifiers: OMI-2 Purifier Tube

J. Syringe: 10 µL syringe, tapered, fixed 23-26s/42/HP needle

K. FID Jet: Capillary Series 530 mm jet (0.011 in ID tip

III. **GC Operating Parameters**

A. Gas Flows: (all measured at 100°C)

1.	Column Inlet Pressure	≈40 psi
2.	Column Flow	≈1.3 mL/min helium
3.	Split Vent Flow:	≈18 mL/min
4.	Split Ratio:	≈15:1
5.	Hydrogen:	≈40 mL/min
6.	Compressed Air:	≈450 mL/min
7.	Auxiliary Gas:	≈45 mL/min

B. Temperatures:

1.	Injection Port:	225°C
2.	Column Oven:	Initial: 100°C hold for 4 min
		Ramp: 2.5°C / min to 240°C hold for 15 min
3.	Detector:	285°C

C. Injection Volume: 1 µL

D. Run Time: ≈82 min / injection

IV. Standard Preparation

When not in use, standards should be stored between -25 to -10°C.

Individually dissolve 25 mg of each standard into 5 mL heptane. Mix well. Transfer to autosampler vials.

V. Sample Preparation

A. Weigh 0.200 - 0.250 g of sample (fat or oil) into a 50-mL round bottom flask.

B. Add approximately 7 mL of 0.5N methanolic sodium hydroxide and a glass bead to the flask.

C. Attach flask to condenser and reflux for 10 minutes.

D. Add 5 mL of boron trifluoride methanol solution through top of condenser

E. Allow to reflux for another 2 minutes.

F. Add 10 mL of heptane through top of condenser.

G. Allow to reflux for another 1 minute.

H. Remove flask from reaction set up.

I. Add 15 mL of saturated sodium chloride solution, stopper, and shake vigorously for 15 seconds.

J. Remove stopper and add more saturated sodium chloride solution to float organic layer into the neck of the flask.

K. When layers have fully separated, transfer a portion of the organic (top) layer to a small test tube.

L. Add a small amount of anhydrous sodium sulfate to the test tube to dry the sample.

M. Cork the test tube and mix well.

N. Allow the sodium sulfate to settle and the solution to become clear.

O. Transfer prepared sample to an autosampler vial.

VI. **Calculations**

A. Identification of Peaks

1. Analyze the standard mixtures of known composition and measure retention times for the constituent esters.

2. Identify the peaks for the sample from these standard retention times.

B. For the general case, in which significant amount of components below C_{12} are absent, calculate the content of a particular constituent (expressed as percent of methyl esters) by determining the percentage represented by the area of the corresponding peak relative to the sum of the areas of all the peaks.

$$\%\,\mathrm{MethylEster} = \frac{\text{peak area of individual component}}{\text{total peak area of all components}} \times 100$$

Appendix B

Thiobarbituric Acid Reactive Substances (TBARS)

I. **Reagents / Materials**

A. Hydrochloric Acid

B. 2-Thiobarbituric Acid

C. Dow Antifoam

D. 1:2 Hydrochloric Acid: Carefully combine 1 part concentrated HCl and 2 parts distilled water. Mix well.

E. 0.02M Thiobarbituric Acid (TBA): Dissolve 0.2882 g thiobarbituric acid in 100 mL distilled water. Mix well.

F. Distilled water

II. **Instrumentation**

A. Spectrophotometer

B. Water Bath

III. **Procedure**

A. Obtain a 250 mL flat-bottomed round flask and place two glass beads inside.

B. Weigh out 10 g of sample, record the weight, and blend with 50 mL of distilled water in a laboratory blender.

C. Carefully transfer the blended sample into the 250 mL flask.

D. Wash the blender with 47.5 mL of distilled water and add to the flask.

E. Add 2.5 mL of 1:2 hydrochloric acid and 3 - 4 drops of Dow Antifoam.

F. Distill the contents of the flask at the highest temperature possible on a hot plate and collect the distillate into a 100 mL beaker.

G. Continue to distill the contents of the flask until you have collected 50 mL of distillate in the beaker.

H. Mix the distillate.

I. Pipette 5 mL of distillate and 5 mL of 0.02M TBA into a scintillation vial and mix well.

J. Prepare a blank by pipetting 5 mL of distilled water and 5 mL of 0.02M TBA into a scintillation vial.

K. Immerse the vials in a 95° C water bath for 35 minutes.

L. Allow the vials reach room temperature by immersing them in cold water for 3 - 5 minutes.

M. Turn on the spectrophotometer by turning the Power Switch/Zero Control (knob on the left side of the instrument) clockwise.

N. Wait 15 minutes for the spectrophotometer to warm up.

O. Set the Wavelength Control (knob on top) to 532 nm.

P. Set the filter lever (small lever at the bottom) to the 340 - 599 nm wavelength position.

Q. Adjust the meter to 0% T with the Power Switch/Zero Control knob. Make sure the sample compartment is empty and the sample cover is closed when you do this.

R. Wash a spectrophotometer test tube twice with a small amount of the blank solution.

S. Fill the test tube with the rest of the blank solution.

T. Wipe off the outside of the test tube to remove dust and fingerprints and place it in the sample compartment.

U. Align the guide mark on the test tube with the guide mark at the front of the sample compartment. Close the lid and adjust the meter to 100% T with the Transmittance/Absorbance Control (knob on the front right side of the instrument).

V. Remove the test tube from the sample compartment and discard the solution.

W. Press the Mode button to switch the instrument from percent transmittance to absorbance.

X. Using the same test tube used for the blank solution, rinse the test tube with small amounts of sample, and read each sample solution recording the absorbance value.

IV. **Calculations**

$$\text{Sample Absorbance} \times 7.8 = \text{TBARS}$$