

**INCLUSION OF BLENDED LIPID SOLUTIONS AS FUNCTIONAL INGREDIENTS TO  
ALTER THE FATTY ACID PROFILE OF BEEF PATTIES**

A Thesis

by

AUSTIN COLE LOWDER

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2009

Major Subject: Animal Science

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Approved by:

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## ABSTRACT

Inclusion of Blended Lipid Solutions as Functional Ingredients to Alter the Fatty Acid Profile of Beef Patties. (August 2009)

Austin Cole Lowder, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Wesley N. Osburn

We hypothesized that beef patties formulated with the addition of a beef fat, plant oil and rosemary extract (antioxidant) blend would increase unsaturated fatty acid content and maintain desirable sensory attributes as compared to 10 and 20% fat control beef patties. Treatment patties were formulated by combining beef trimmings (6% fat) with a lipid blend mixture (4% or 14% addition, respectively) containing 57% beef tallow, 0.3% rosemary extract and 43% of either high oleic safflower oil (SO), olive oil (OO), or corn oil (CO) to achieve a total fat content of 10 or 20%. Treatment patties were similar to control patties for lipid oxidation at 0 and 3 d of refrigerated (2°C) storage and up to 56 d of frozen (-10°C) storage. Cooked lipid blend patties at 10 or 20% fat content were similar to or higher, respectively, than control patties for juiciness and were no different for other sensory attributes evaluated. At 10 and 20% fat levels, oleic acid (18:1) in cooked SO patties (46.1 and 50.3%, respectively) and OO patties (43.8 and 48.1%, respectively) was higher than the control (37.3 and 37.6%, respectively). Unsaturated to saturated fatty acid ratios at the 10 and 20% fat levels were higher in SO (1.37 and 1.60, respectively) and CO (1.40 and 1.48, respectively) patties than the control (0.97 and 0.94, respectively). The incorporation of nutritionally enhanced lipid blends increased unsaturated fatty acid content and maintained desirable sensory attributes of beef patties while suppressing lipid oxidation.

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## TABLE OF CONTENTS

		Page
ABSTRACT .....		iii
ACKNOWLEDGEMENTS .....		iv
TABLE OF CONTENTS.....		v
LIST OF TABLES .....		viii
CHAPTER		
I	INTRODUCTION AND REVIEW OF LITERATURE .....	1
	Introduction.....	1
	Fatty acid structure and nomenclature .....	1
	Impact of fat on the nutritive value of meat.....	2
	Fatty acid composition of meat .....	4
	Components of meat quality .....	5
	Manipulating meat composition.....	6
	Manipulating fatty acid composition and the effects on meat quality .....	8
	Sensory attributes .....	9
	Shelf life .....	10
	Reformulation of meat products to alter fatty acid composition ....	12
	Types of oils/fat.....	12
	Fat replacement technology .....	13
	Effects of reformulating processed meat products with enhanced fatty acid composition .....	15
	Influence on processing and quality characteristics.....	15
	Potential health benefits and challenges .....	18
	Summary of literature.....	20
II	MATERIALS AND METHODS .....	22
	Lipid manufacture .....	22
	Raw material preparation.....	23
	Control patty manufacture.....	23
	Treatment patty manufacture .....	23
	Sample packaging .....	24
	Refrigerated retail and frozen beef patty shelf life study.....	24

CHAPTER	Page
Lipid blend objective color.....	25
pH and proximate composition.....	25
Lipid oxidation.....	26
Beef patty objective color determinations.....	26
Fatty acid composition.....	27
Allo-Kramer shear force determination and dimensional changes.....	28
Trained sensory panel analysis.....	28
Statistical analysis.....	29
 III RESULTS AND DISCUSSION.....	 31
Introduction.....	31
Materials and methods.....	32
Lipid manufacture.....	32
Raw material preparation.....	33
Control patty manufacture.....	33
Treatment patty manufacture.....	35
Sample packaging.....	35
Refrigerated retail and frozen beef patty shelf life study.....	36
Lipid blend objective color.....	36
pH and proximate composition.....	37
Lipid oxidation.....	37
Beef patty objective color determinations.....	37
Fatty acid composition.....	38
Allo-Kramer shear force determination and dimensional changes.....	38
Trained sensory panel analysis.....	39
Statistical analysis.....	40
Results and discussion.....	40
Objective color of lipid blends.....	40
Proximate composition.....	41
Comparison of TBA values during refrigerated storage.....	41
Comparison of TBA values during frozen storage.....	45
Refrigerated raw patty color determination.....	47
Frozen raw patty color determination.....	47
pH determination.....	50
Cooked internal color, cook yield, Allo-Kramer shear, and dimensional changes.....	50
Sensory evaluation.....	52
Fatty acid composition.....	55
Conclusions.....	56
 IV CONCLUSIONS.....	 59
REFERENCES.....	61

	Page
APPENDIX A.....	66
APPENDIX B.....	67
APPENDIX C.....	68
APPENDIX D.....	74
APPENDIX E.....	77
APPENDIX F.....	79
APPENDIX G.....	81
VITA.....	83

## LIST OF TABLES

TABLE		Page
1	Formulation weights (kg) for manufacture of beef patties containing a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) .....	34
2	Least squares means for L*, a*, and b* values of lipid blends formulated with beef tallow (57%) and mono- or polyunsaturated oils (43%) .....	42
3	Main effect means (fat level and lipid blend treatment) for proximate composition of raw and cooked beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) .....	43
4	Two-way interaction (lipid blend treatment x storage day) least squares means for TBA values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at 0, 3, 6, 9 days of storage.....	44
5	Two-way interaction (lipid blend treatment x storage day) least squares means for TBA values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at days 0, 7, 14, 28, 42, and 56 of storage.....	46
6	Two way interaction (lipid blend treatment x storage day) least squares means of L*, a*, and b* values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at days 0, 3, 6, and 9 of refrigerated storage .....	48
7	Two way interaction (lipid blend treatment x storage day) least squares means of L*, a*, and b* values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at days 0, 7, 14, 28, 42, and 56 of frozen storage.....	49
8	Least squares means for cook yield, shear values, cooked CIE L*, a* and b* values and dimensional changes of beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) ...	51



TABLE		Page
9	Least squares means of descriptive attribute sensory panel scores (combined) for textures, aromatics, basic tastes, mouthfeels, aftertastes, and after mouthfeels of cooked beef patties formulated with a mixture of beef tallow (57%) and mono- or polyunsaturated oils (43%) at 10% and 20% fat levels .....	54
10	Least squares means for fatty acid composition of lipid blends formulated with beef tallow (57%) and mono- or polyunsaturated oils (43%) .....	57
11	Least squares means for fatty acid composition of cooked beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) .....	58

## CHAPTER I

### INTRODUCTION AND REVIEW OF LITERATURE

#### Introduction

The increased awareness of the importance of diet to human health has prompted many consumers to carefully consider the foods they purchase. Although there are many factors that influence the nutritional quality of a given food product, great attention is often paid to the total amount of fat present and its fatty acid composition. This is because both of these attributes have been implicated as contributing factors to certain cancers and cardiovascular diseases. Muscle foods have been criticized for their high total fat content and individual fatty acid content, specifically their low polyunsaturated (PUFA) to saturated (SFA) fatty acid ratios (PUFA:SFA). It is for these reasons that methods of altering the fatty acid profile in muscle foods continues to gain interest.

#### Fatty acid structure and nomenclature

Akoh (1998) describes the structure and nomenclature of food lipids. Lipids within muscle food systems generally consist of 12 – 24 carbons atoms with a nonpolar methyl group (CH<sub>3</sub>) at one end of the chain and a polar carboxylic acid group (COOH) at the other. If none of the carbons are double bonded together, the fatty acid is said to be saturated with hydrogens; it is put into the class of saturated fatty acids (SFA). The presence of double bonds makes the fatty acid unsaturated. Unsaturated fatty acids (UFA) can be classified as either monounsaturated (MUFA), containing a single double bond, or polyunsaturated (PUFA), containing multiple double bonds.

The use of several different nomenclature systems complicates the discussion of

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fatty acids. There are several that will commonly be used in this text. Shorthand nomenclature is used to identify the number of carbons a fatty acid has followed by the number of double bonds. An 18 carbon fatty acid containing one double bond would be identified using shorthand as 18:1, for example. Trivial names are given to individual fatty acids, such as oleic acid (18:1) or stearic acid (18:0). The system used to denote double bond placement will be the n minus system. This nomenclature identifies the position of the first double bond from the methyl end of the fatty acid by subtracting the number of carbons to the first double bond from the total number of carbons (n). The first double bond in linoleic acid (18:2), for example, is six carbons from the methyl end. This would be identified in short hand as 18:2n-6. Fatty acids can often be classified simply by their n minus designation as this will indicate their eventual purpose within the body.

The physical characteristics of individual fatty acids are determined by chain length and presence of double bonds. Fatty acid melting point is increased slightly as the number of carbons increases. Palmitic acid (16:0), for example, has a melting point of 63-64°C while stearic acid (18:0) has a melting point of 70°C. Increasing double bonds has a much greater effect on melting point. The melting point for oleic acid (18:1) is 18°C, far less than the 70°C melting point of 18:0. Some bacteria, like those in the rumen, can hydrogenate fatty acids and create double bonds in the *trans* configuration. This means that the hydrogens on either end of a double bond are on the same side of the molecule. Melting point is less affected by *trans* double bonds than *cis* double bonds as they put a much smaller kink in the molecule (Akoh 1998).

### **Impact of fat on the nutritive value of meat**

Intake of lipids from dietary sources is generally in the form of a triacylglycerol (TAG). This molecule consists of three fatty acids bound by an ester linkage to a glycerol structure. TAG represent most of the stored lipids in the body. Phospholipids (PL)

represent the second major group of lipids. These are lipid structures with an attached phosphate group. Lecithin, an emulsifier important to the functions of bile, and sphingomyelin, an integral component of the myelin sheaths surrounding nerve axons, are both notable phospholipids. Any other classes of lipids from dietary sources are generally present in insignificant amounts. Food lipids contain practically no free fatty acids (Cichon 2003).

Food lipids are used for a wide variety of functions in the body, not all of which are known. In very basic terms of metabolic energy lipids provide 9 kcal/g, where proteins and carbohydrates provide 4 kcal/g. The human body has the ability to synthesize many fatty acids through enzymatic activity that either increases carbon chain length (elongation) or remove inserts double bonds (desaturation). The n-6 and n-3 fatty acid families cannot be synthesized by humans, but are present in plant dietary sources. The fatty acids in these families are termed essential fatty acids (EFA) and they are used by the body to synthesize various steroid hormones, maintain cell membranes and regulate many cellular functions (Cichon 2003).

The high amount of SFA in beef is often cited as a health concern for consumers because they tend to increase total plasma cholesterol and low density lipoprotein (LDL) cholesterol, both risk factors for cardiovascular disease (Mattson and Grundy 1985). It should be noted that stearic acid (18:0), which represents about 30% of SFA in beef, has little effect on the levels of plasma lipoproteins (Yu and others 1995). Evidence collected in a compilation of studies by Kris-Etherton and Yu (1997) shows oleic acid (18:1), which is the single most prominent fatty acid in beef, to lower both total cholesterol and LDL cholesterol. The same review shows the major PUFA in beef, linoleic acid (18:2) to lower both total cholesterol and LDL cholesterol while increasing amounts of HDL cholesterol.

One of the most beneficial fatty acids present in beef is conjugated linoleic acid (CLA). Conjugated linoleic acids are a series of isomers of linoleic acid (Pariza and others

2001). The two isomers of most interest, *cis*-9, *trans*-11 and *trans*-10, *cis*-12, have been shown to have beneficial health effects. CLA isomer *cis*-9, *trans*-11 is believed to have protective properties from the effects of cancer (De la Torre and others 2006) and atherosclerosis (Lee and others 1994) effects, while CLA *trans*-10, *cis*-12 has shown anti-obesity effects (Park and others 1997).

The presence of n-6 fatty acids, such as linoleic acid (18:2n-6), and n-3 fatty acids, such as linolenic acid (18:3n-3), greatly affects the nutritive value of meat. Both fatty acids reduce total cholesterol and LDL cholesterol, but n-3 fatty acids provide a host of other health benefits, including being anticarcinogenic, antithrombotic, and antiarrhythmic (Leaf and others 2003, Calder 2004). The chain elongation and further desaturation necessary for the body to utilize 18:2 and 18:3 occurs through the same enzymatic pathway (Calder 2004). Because of this, the formation of longer chain 18:3 derivatives in the human body is inefficient (Pawlosky and others 2001; Burdge and others 2002). The most efficient source of long chain n-3 fatty acids are fish and fish oils, however, there is concern over the low consumption in western diets and questionable sustainability of these sources (Williams and Burdge 2006). Because of a physiological state conducive to platelet aggregation, cancerous cell proliferation and inflammation (Simopoulos 2002) caused by high n-6 diets, n-6:n-3 ratios above 4 are considered a risk factor for cancer, coronary heart disease and sudden cardiac arrest, and attention is placed on increasing the amount of n-3 fatty acids in the diet. Beef contains a low n-6:n-3 ratio, usually less than 3:1 (Scollan and others 2006), while pork is shown to have a higher range of 7.57 – 12.23 (Enser and others 1996; Wood and others 2004a).

### **Fatty acid composition of meat**

In beef, intramuscular fat is composed, on average, of 45-48% SFA, 35-38% monounsaturated fatty acids (MUFA), and around 5% PUFA (Scollan and others 2006).

The most prevalent SFA are myristic acid (14:0), palmitic (16:0) and stearic (18:0), while MUFA are almost entirely represented by oleic acid (18:1) and its isomers, and PUFA are mostly made up of linoleic acid (18:2) and  $\alpha$ -linolenic acid (18:3). Also present in beef are several isomers of conjugated linoleic acid (CLA), with the cis-9, trans-11 isomer accounting for 70% of all CLA (Dannenberger and others 2005).

It is important to acknowledge the differences in fatty acid composition between species. Enser and others (1996) used retail steak or chop samples from similar locations (loin) on beef, pork, and lamb carcasses to determine total adipose tissue amount and individual fatty acid content. The results showed that for the whole steak or chop beef and lamb samples contained very low PUFA:SFA ratios (0.07 and 0.09, respectively), while pork displayed a higher ratio of 0.61. This discrepancy between beef and lamb (ruminants) and pork (non-ruminant) PUFA:SFA ratios is because of biohydrogenation, a process that hydrogenates unsaturated fatty acids in the rumen (Wood and others 1999). Because of this process, long chain n-3 PUFA tend to show much greater deposition in pork than beef or lamb. Determination of n-6:n-3 ratios showed lower values for beef and lamb (2.22 and 1.28, respectively) than pork (7.57).

### **Components of meat quality**

The direct effects of fatty acid composition on meat quality are limited to shelf life and flavor. Lipid oxidation of unsaturated fatty acids explains the effect fatty acids can have on shelf-life. Lipid oxidation is a process by which unsaturated fatty acids are converted to lipid free radicals. These free radicals react with molecular oxygen and other unsaturated lipids to form lipid hydroperoxides. Eventually, byproducts from these reactions will cause sensory attributes decrease and nutritional value to decline. This condition in food systems is termed rancidity. Meat products seem to be susceptible to lipid oxidation because of the availability of iron to initiate the process (Rhee and Ziprin

1987). Continued exposure of muscle to oxygen causes the color to change over time, as the muscle pigment oxidizes, from red (oxymyoglobin) to brown (metmyoglobin). This reaction tends to proceed in parallel with rancidity and some lipid oxidation byproducts have been shown to promote oxidation in muscle pigment. Although meat containing a higher degree of unsaturated fatty acids would likely increase in rate of oxidation, pasture fed cattle tend to have more antioxidant in the form of  $\alpha$ -tocopherol, while cattle fed lipid supplements, such as linseed oil or fish oils, do not (Scollan and others 2006).

Characteristic meat flavor is produced during cooking, when volatile compounds are released as a function of two mechanisms: 1) Maillard reaction, and 2) thermal degradation of lipids (Mottram 1998). The Maillard reaction takes place when amino acids and reducing sugars are combined under heat; this reaction produces flavors characteristic of all cooked meat. Volatiles derived from lipid degradation produce the species specific flavors found in different muscle foods (Mottram 1998). Also important to meat flavor are the unsaturated fatty acids found in the phospholipids present in animal cells (Mottram 1998). Alteration of the fatty acid profile to include more unsaturated fatty acids could change the type and amount of volatiles released during cooking, which would alter flavor and aroma (Scollan and others 2006). Campo and others (2003) employed a trained sensory panel to study the flavor of individual fatty acids alone or in combination with cysteine (amino acid) and ribose (reducing sugar), the principal reactants of the Maillard reaction. It was found that oleic acid (18:1n-9), linoleic acid (18:2n-6) and  $\alpha$ -linolenic acid (18:3n-3) all produced meaty flavors in the presence of the amino acid and sugar and that  $\alpha$ -linolenic acid (18:3n-3) produced fishy flavors both individually and in combination with the Maillard reactants.

### **Carcass modification to alter the fatty acid composition of meat**

Efforts to alter the profile of fatty acids present in beef cattle preharvest have resulted in a multitude of different approaches. Often the most convenient approach is the feeding of grass or silages which are rich in n-3 fatty acids. Neurnberg and others (2005) compared two breeds (German Simmental and German Holstein) of beef cattle fed concentrates to those that were pasture fed and subsequently placed on a concentrate diet supplemented with grass silage and linseed oil. The grass based diet was able to increase ( $P < 0.05$ ) the amount of n-3 PUFA in the loin muscle of both breeds while having no effect on the amounts of n-6 PUFA present, effectively reducing the n-6:n-3 ratio to 2.0 and 1.9 for the grass fed cattle compared to 8.3 and 6.5 for the grain fed. The authors also saw a significant increase ( $P < 0.05$ ) of 18:1 in the grass fed cattle as well as a significantly larger ( $P < 0.05$ ) amount of CLA isomer *cis*-9, *trans*-11. Varela and others (2004) compared cattle fed on a pasture finishing system to those finished with a maize silage and concentrate system. Although no change ( $P > 0.05$ ) in the amount of  $\alpha$ -linolenic acid (18:3n-3) present in the *Longissimus* intramuscular fat was seen, a decrease ( $P < 0.05$ ) in n-6:n-3 ratio displayed by the pasture fed cattle (2.67) versus the concentrate fed cattle (4.06) was.

Supplementation of oil in the diet has also shown to be an effective method of altering an animal's fatty acid content, as shown by Scollan and others (2001). The authors conducted a study that used a 60:40 forage:concentrate diet supplemented with either palmitic acid (16:0) as a control, linseed oil, which contains high amounts of 18:3n-3, fish oil, or a combination of linseed oil and fish oil. As noted earlier, fish oil, which contains eicosapentanoic acid (20:5n-3) and docosahexanoic acid (22:6n-3), is a more efficient way to provide n-3 fatty acids to humans as these are the eventual products that  $\alpha$ -linolenic acid (18:3n-3) will provide through chain elongation and desaturation. The linseed oil and linseed oil-fish oil mixtures were able to increase ( $P < 0.05$ )  $\alpha$ -linolenic acid



(18:3n-3) over cattle fed the control diet, while all treatments showed a reduction of linoleic acid (18:2n-6) in triacylglycerols (TAG). The long chain PUFA present in fish oil were not detected in the TAG or subcutaneous adipose tissue, suggesting a high susceptibility to biohydrogenation. All three treatments displayed a decrease in n-6:n-3 ratio. Noci and others (2005) undertook a study to determine the effects of different amounts of sunflower oil (SFO) in diets fed to crossbred heifers. Biohydrogenation of 18:2n-6 to 18:0 in the rumen has been shown to produce CLA isomers as intermediates (Harfoot and Hazlewood, 1988). While the increased amounts of SFO were shown to significantly increase ( $P < 0.05$ ) the amount of CLA isomer *cis*-9, *trans*-11 present in the intramuscular fat of the *Longissimus dorsi* muscle, they also had the effect of increasing ( $P < 0.05$ ) the n-6:n-3 ratio beyond recommended levels.

As previously noted, rumen biohydrogenation is one of the principal obstacles to the alteration of fatty acid composition in beef cattle. A novel approach to overcome this obstacle is protection of the lipid supplement via protein encapsulation or chemical protection. This approach was used by Scollan and others (2003) in a study that used grass fed cattle supplemented with concentrate treatments containing varying amounts of a ruminally protected lipid supplement (PLS) containing high values of  $\alpha$ -linolenic acid (18:3n-3) fatty acids. Feeding the PLS treatments tended to reduce the amount of SFA while increasing ( $P < 0.05$ ) overall PUFA and 18:3n-3 in intramuscular fat, showing that the PLS treatments were afforded a high degree of protection from the biohydrogenating action of the rumen.

Enser and others (2000) fed pigs a test diet supplemented with crushed whole linseed, which is high in  $\alpha$ -linolenic (18:3) acid, and a control diet. The test diet was effective in producing porcine intramuscular fat with a PUFA:SFA value of 0.4, which is within recommended levels. Lower n-6:n-3 fatty acid ratios were seen in intramuscular fat from pigs fed the test diet (5) than the control diet (8-9).

A crushed linseed diet was also used to increase n-3 PUFA by Kouba and others (2003). The linseed diet produced pigs a favorable n-6:n-3 ratio in intramuscular fat of 3.11 versus the control ratio of 8.71 after 100 d, but the PUFA:SFA ratio for both diets was slightly lower than the recommended 0.4 (0.38 and 0.31, respectively).

### **Manipulating fatty acid composition and the effects on meat quality**

Research has shown that it is possible to alter fatty acids profiles of animal fats by dietary strategies. The ability of meat from these animals to maintain oxidative and color stability, as well as exhibit desirable sensory attributes is important to determine whether or not it can be successfully offered to consumers.

#### **Sensory attributes**

Vatanever and others (2000) used oil supplements high in n-3 PUFA to supplement the diet of Holstein-Friesian and Welsh Black cattle fed for 120 d. Cattle were subsequently harvested and steaks were cut from the *longissimus* while minced beef burgers were manufactured from the *infraspinatus*, *supraspinatus*, and *triceps brachii*. Steaks were used for retail display and either pulled for sensory analysis after 5 d or used for lipid oxidation testing after 4, 8, or 11 d. Trained sensory panel scores showed the steaks from the fish oil treatment to have higher ( $P < 0.05$ ) values for toughness, rancidity, and fishiness than the linseed oil and linseed oil/fish oil treatments.

Fatty acid analysis of Limousin and Belgian Blue cattle by Raes and others (2003) compared to imported Argentine and Irish cattle showed the former two breeds had higher overall PUFA amounts and n-6:n-3 ratios. This suggests that the Limousine and Belgian Blue cattle were grain fed, while the latter two breeds were grass fed. Trained sensory panel and gas chromatograph – mass spectrometry (GC-MS) analyses discovered higher ( $P < 0.05$ ) flavor intensity among the grass fed cattle.

Neurnberg and others (2005) conducted sensory analysis on steaks from cattle fed either a grass based diet or a concentrate based diet. The only sensory differences detected between steaks from concentrate fed cattle and steaks from grass fed cattle were overall liking (16.2 and 12.2, respectively) and fishy flavor (1.7 and 4.3, respectively). Fishy flavor, which scored higher ( $P < 0.05$ ) in the grass fed cattle, is an indicator of rancidity and a known indicator of the presence of 18:3n-3 (Campo and others 2003).

Chops and bacon from pigs with increased n-3 PUFA (Sheard and others 2000) were not found to have any differences ( $P < 0.05$ ) from control products as determined by a trained sensory panel. Sausages produced from the pigs with increased n-3 PUFA were rated higher ( $P < 0.05$ ) than the control sausages for overall liking.

### **Shelf life**

Thiobarbituric acid reactive substances (TBARS) assays, which are a measurement of lipid oxidation, conducted by Vatansever and others (2000) on retail steak samples from cattle fed oils high in n-3 PUFA showed increased TBARS values over time for all overwrapped steaks. Only a fish oil treatment, high in long chain PUFA, reached the threshold TBARS value of 1 mg malonaldehyde per kg of meat. This is the point at which rancidity can be detected by consumers (Younathan and Watts 1959). For burgers used in the same study, the index value of 1 was passed by the fish oil treatment at 3 d and by the linseed and linseed/fish oil treatments at 10 d.

Crossbred steers fed a grass diet, concentrate diet (CON), or a varying mix of the two diets yielded steaks that were subsequently aerobically packaged or packaged within a modified atmosphere (MAP) and tested for color and lipid oxidation (O'Sullivan and others 2003). Under MAP packaging, the grass fed cattle produced steaks that were higher ( $P < 0.05$ ) for  $a^*$ (redness) values on days 4 to 12 than all other diets.

Metmyoglobin, the brown pigment observed in discolored beef, was analyzed for aerobic

and MAP packaged steaks. Higher ( $P < 0.05$ ) metmyoglobin levels were seen for steaks from the CON group of cattle for days 6 to 12 (MAP) and lower metmyoglobin levels in steaks from cattle fed the grass diet for days 6 to 12 (aerobic). Subjective color analysis by sensory panel showed preferences for steaks from cattle fed the grass fed diet under MAP as it had a redder color. Lipid oxidation as determined by TBARS analysis showed higher ( $P < 0.05$ ) levels for steaks from cattle fed the concentrate dietary treatment compared to the grass fed group and all combinations in between.

Yang and others (2002) evaluated both pasture fed cattle and grain fed cattle supplemented with  $\alpha$ -tocopherol. Control and supplemented pasture fed and control and supplemented concentrate fed cattle were estimated to intake 2200, 4700, 300 and 2800 International Units (IU)/head/day, respectively. The authors found that supplementation raised the tocopherol levels of the grain fed cattle to near those of the pasture fed cattle. Steaks from the pasture fed cattle, which were higher in  $\alpha$ -linolenic (18:3n-3) and overall PUFA, were more susceptible to oxidation and has less red color than the antioxidant supplemented grain fed cattle. This evidence would seem to indicate that  $\alpha$ -tocopherol content is very important to the oxidative and color stability of meat.

The pigs fed by Enser and others (2000) to have increased amounts of n-3 PUFA were used to produce pork chops, liver, bacon and sausages by Sheard and others (2000), which were assessed for shelf life attributes. Chops from the test diet displayed a lower ( $P < 0.05$ ) TBA value than the control diet (0.051 and 0.061, respectively), while liver, bacon and sausages saw differences in sensory attributes from dietary treatment. Through 8 d of testing chops, bacon and sausages showed no change in color measurements. Overall, products produced from pigs with increased n-3 PUFA in this study did not show any detrimental effects to shelf life attributes.

Lipid oxidation determination on pigs fed either a crushed linseed diet or control diet (Kouba and others 2003) for 100 d showed that muscle from pigs on the linseed diet

was more susceptible ( $P < 0.05$ ) to oxidation than those fed a control diet, although overall values were low ( $< 0.2$  mg malonaldehyde/kg meat).

### **Reformulation of meat products to alter fatty acid composition**

The development stages of processing offer an opportune time to alter the composition of meat products. Products can be formulated to meet specific nutritional goals, such as reducing fat content to limit the calories offered by a product. Low fat meat products, however, are known to present challenges related to processing characteristics (Keeton 1994) and sensory attributes. Also, fat reduction in processed meats may not be able to limit dietary caloric intake from fat to recommended levels (WHO 2003). Instead of focusing on a reduction in lipid intake, reformulation can be used to create products with specific fatty acid compositions that are potentially beneficial to human health.

#### **Types of oils/fat**

Vegetable oils are a convenient and readily available source of unsaturated fatty acids to incorporate into processed meat products. They can be high in MUFA (Olive oil, high oleic safflower oil, high oleic sunflower oil) or PUFA (soybean oil, sunflower oil, safflower oil, corn oil, cottonseed oil, linseed oil) and are cholesterol free.

Bloukas and others (1997) used olive oil as a replacement for pork fat in sausages because of its high concentration of MUFA and natural antioxidant capacity. Olive oil has also shown to be antithrombotic and preventative of LDL oxidation, as noted by Luruena-Martinez and others (2004) who used olive oil and hydrocolloids to reduce fat levels in frankfurters. Park and others (1989, 1990) were able to incorporate high oleic sunflower oil into frankfurters to increase MUFA content.

Incorporation of PUFA is often easily achieved through the use of vegetable oils. Paneras and Bloukas (1994) and Paneras and others (1998) were both successful in

raising the amount of PUFA through the use of cottonseed and corn oils, while also raising the n-6:n-3 ratio, noted earlier as being a risk factor for cancer and cardiovascular diseases. Work (Bishop and others 1993) with bologna also used corn oil, in a pre-emulsified form to replace pork fat. A general increase in PUFA might not be the best course of action as it can often raise the n-6:n-3 ratio; oils higher in n-3 PUFA could provide a more nutritionally beneficial fatty acid profile. Pelsler and others (2007) manufactured Dutch style sausages enriched with canola oil or encapsulated flaxseed oil, seeing an increase in PUFA:SFA ratio as well as an decrease in n-6:n-3 ratio in both treatments compared to the controls. Another oil high in n-3 fatty acids, linseed oil, was used by Ansorena and Astiasaran (2004) in dry-fermented sausages to greatly reduce the n-6:n-3 ratio from 14.1 in the control sausages to 1.7 in the linseed oil treatment, while maintaining increased PUFA:SFA ratios.

Use of fish oils, which are high in long chain n-3 PUFA that are likely to be more efficiently utilized by the human body (Calder 2004), as beef or pork fat substitutes has been documented by Pelsler and others (2007). The authors observed that batches manufactured with fish oil yielded a lower n-6:n-3 ratio than other treatments. Kolanowski and Laufenberg (2006) noted in a review on the subject that although fish oil is often associated with fishy aromas and a high susceptibility to lipid oxidation, these obstacles can be overcome through oil refinement and deodorizing treatments and employment of antioxidants.

### **Fat replacement technology**

The high amounts of unsaturated fatty acids in liquid oils, which causes very low melting points and high susceptibility in oxidation, lead to challenges when incorporating them into processed meat products. Care must be taken to ensure that the oil is included in a droplet form to prevent product and quality losses. The work of Whiting (1987) shows

that gel strength, water exudation, and fat loss are all correlated with degree of unsaturation in the fat of a comminuted product.

Liquid oils (ground nut oil and maize oil) were used by Dzudie and others (2004) for incorporation into beef patties. Shiota and others (1995) also incorporated liquid oils (soybean, palm, high-oleic palm) into beef patties. Work on fermented sausages (Bloukas and others 1997) and salami (Severini and others 2003) also included liquid oils.

Oils that are difficult to stabilize can be pre-emulsified using an emulsifier, usually a non meat protein, to improve the system's fat binding ability by setting the oil in a protein matrix or immobilizing it (Jimenez-Colmenero, 2007). The emulsion is made prior to product manufacture and added as a fat, and can be easily enhanced by adding an antioxidant as protection against lipid oxidation. Ansorena and Antiasaran (2004), Bloukas and Paneras (1993), Paneras and Bloukas (1994), and Pelsler and others (2007) all cite the method of Hoogenkamp for developing the pre-emulsion. Hoogenkamp's method involves mixing eight parts hot water with one part sodium caseinate or soy protein isolate for 2 minutes, after which this mixture is emulsified with 10 parts oil for 3 minutes (Jimenez-Colmenero, 2007). Mourtzinou and Kiosseoglou (2005) describe a method used to create a corn oil emulsion with isolated soy protein as the emulsifier. Emulsions were prepared by dissolving isolated soy protein in a phosphate buffer at pH 6.5 followed by dropwise addition of corn oil to the continuous phase.

Solid fats containing mostly saturated fatty acids or partially hydrogenated oils can be incorporated into processed meats as they have higher melting points and are more stable at ambient temperatures. Babji and others (1998) used partially hydrogenated palm oil to replace beef fat in beef patties and Liu and others (1991) used partially hydrogenated corn, cottonseed, palm, peanut, and soybean oils in beef patties. While the use of partial hydrogenation in oils affords more consistency and stability, the process results in the formation of *trans* fatty acids and an increased degree of saturation in

triacylglycerol (TAG) fatty acids. This leads to increases in plasma cholesterol concentration and LDL cholesterol and decreases in HDL cholesterol (Kris-Etherton and Yu 1995; Simopoulos 2002). Interesterification allows for chemical or enzymatic alteration of a TAG structure to increase melting point and stability without increasing saturation or adding *trans* double bonds. Interesterified oils have been used successfully in frankfurters (Vural and others 2004) and salami (Javidipour and Vural 2002).

### **Effects of reformulating processed meat products with enhanced fatty acid composition**

The various technologies developed have allowed researchers to influence the fatty acid profile of many different types of processed meat products. Attention must be paid, however, to the effects that increasing the amount of unsaturated fatty acids has on processing characteristics and quality issues.

#### **Influence on processing and quality characteristics**

When Bloukas and others (1997) replaced up to 20% of pork fat in frankfurters with olive oil as a liquid and a pre emulsified fat, they found that the olive oil treatments had higher lightness and yellowness values compared to the control. The liquid olive oil treatments displayed lower weight losses than the control; however they had higher TBA values and were determined to have an unacceptable appearance. When incorporated in pre-emulsified form, the olive oil treatments had lower TBA values than the control, but had greater weight losses. The authors concluded that 20% of pork fat could be replaced by olive oil in a pre-emulsified form, using isolated soy protein as an emulsifier.

In a study that replaced pork fat with olive oil and added locust bean/xanthan gum (hydrocolloids) in frankfurters (Luruena-Martinez and others 2004) it was shown that treatment samples with olive oil were lower for hardness and higher for adhesiveness than



the control according to texture profile analysis. However, the replacement of pork fat with olive oil, when combined with hydrocolloids, yielded lower cook losses, better emulsion stability and lower jelly and fat separation. Also, olive oil replacement did not affect overall acceptability.

Park and others (1989), in using high oleic acid sunflower oil to increase MUFA content in beef/pork frankfurters, were able to increase ( $P < 0.05$ ) monounsaturated fatty acid to saturated fatty acid (MUFA:SFA) ratios by 468% over the control. Despite increases in low melting point fats, the frankfurters experienced little weight loss during heating and were not significantly different for most sensory attributes. The authors suggested that the total fat content, rather than amount of unsaturated fatty acids, had far more influence in sensory and texture scores.

Bologna (Bishop and others 1993) containing 20% pre-emulsified corn oil and 10% pork fat was produced and analyzed compared to control bologna. Cooking yield and TBA values were not affected by the addition of corn oil, even though it is high in PUFA. Higher ( $P < 0.05$ ) purge loss values were seen from the samples with corn oil (2.23%) than control (0.30%). The authors believe the higher purge values were due to the oil being liquid at refrigeration temperatures, which allows it to move more freely within the protein matrix. Hunter L, a and b scores were determined on control and treatment bologna. Bologna with corn oil displayed higher L values, which indicate lightness, and lower a values, which indicate redness. Firmness for the control bologna was higher than that of the corn oil bologna. Sensory evaluation showed no differences between treatment and control for flavor, texture or juiciness.

In beef patties containing ground-nut or maize oils (Dzudie and others 2004), the greatest oxidative and microbial stability was seen in patties formulated with maize oil and low levels of ginger and basilica oils as they displayed the lowest TBA values (0.12 and 0.11 mg/kg meat, respectively) and microbial loads. Cooking losses, however, were

greater ( $P < 0.05$ ) for patties manufactured with ground-nut and maize oils (35.85 and 36.90%, respectively) compared to those manufactured with beef fat (32.65%). Softness, as determined by textural analysis was greater for patties manufactured with vegetable oils.

Beef patties manufactured with different liquid oils and liquid fats by Shiota and others (1995) were shown to have decreasing ( $P < 0.05$ ) texture and aroma scores by a trained sensory panel as the amount of unsaturated fats (high oleic palm oil, soybean oil) increased. Use of added beef tallow as a fat source in the beef patties increased aroma scores as fat level increased and increased texture, taste and overall acceptability scores up to 30% fat level. In sensory testing of sausages manufactured with various oils at differing fat levels, also conducted by Shiota and others (1995), the more solid fats (beef tallow, palm oil) yielded decreased ( $P < 0.05$ ) texture and aroma scores as their levels increased.

Sausages (Pelsler and others 2007) were manufactured with canola oil or flaxseed oil, both high in 18:3n-3, and fish oils, high in 20:5n-3 and 22:6n-3. It was observed that treatments with canola oil, due most likely to its high amount of tocopherols, remained similar to the control for peroxide values (oxidation) while treatments with flaxseed oil tended to increase in peroxide value due to the high amounts of 18:3n-3, which is highly susceptible to lipid oxidation. Fish oil treatments were also high for peroxide values, even when incorporated as encapsulated oil, due to the high degree of unsaturation in its main fatty acids.

Dry-fermented sausages containing linseed oil, high in 18:3n-3, substituted for 25% of pork fat were higher for TBA values than control sausages, but were still under the threshold value of 1.0 (Ansorena and Antiasaran 2004). Inclusion of a butylated hydroxytoulene (BHT) and butylated hydroxyanisole (BHA) antioxidant combination in the

formulation reduced all indicators of oxidation to similar to ( $P > 0.05$ ) or lower ( $P < 0.05$ ) than the control.

Beef patties formulated with various hydrogenated vegetable oils (corn, cottonseed, palm, peanut and soybean) by Liu and others (1991) were tested by a trained sensory panel. Panelists found that treatments manufactured with corn or palm oil were not significantly different from the control for overall acceptability. These treatments were also similar ( $P > 0.05$ ) to the control for cooking losses.

The use of interesterified oils and sugarbeet fiber to augment beef fat in frankfurters did not lead to any significant changes in appearance, color, flavor, texture or sensory scores compared to a control frankfurter (Vural and others 2004). Levels of oleic acid increased due to use of interesterified olive oil at 6 and 10% (47.2 and 45.2%, respectively), while use of interesterified cottonseed oil increased levels of linoleic acid. All treatments that incorporated interesterified oils and sugarbeet fiber also had greater water holding capacity than control frankfurters. Similar studies conducted with interesterified oils and sugarbeet fiber in Turkish-type salami also showed no significant differences in color, flavor or texture between treatment and control groups (Javidipour and Vural 2002; Javidipour and others 2005). Use of interesterified olive oil at 10% significantly increased the amount of oleic acid to as much as 58.97% of total fatty acid composition, while lowering the SFA:UFA ratio to 0.44.

### **Potential health benefits and challenges**

The current status of muscle foods as staples in the western diet affords the meat industry a great opportunity to take a step into the ever expanding market of functional foods, a segment which it has been slow to enter. Current WHO dietary fat guidelines state that dietary fat should account for between 15% and 30% of total energy, while no more than 10% of total dietary energy should be from saturated fat, 6-10% should come

from PUFA (n-6, 5-8%; n-3, 1-2%), and approximately 10-15% of total dietary energy should be from MUFA (WHO 2003). There exists sufficient evidence that muscle foods, whether produced by dietary means or processing techniques can be tailored to fit these guidelines, as well as fit recommended ratios of PUFA:SFA (0.4-1.0) and n-6:n-3 fatty acids (<4) (Wood and others 2004b). The industry is currently in a position to use genetic, dietary or processing means to tailor products to offer the health benefits associated with certain qualitative aspects of dietary fat to consumers in a familiar form.

While it has been shown numerous times that it is possible to qualitatively alter the fatty acid composition of meat products through dietary and processing means, products that claim to offer health benefits should contain large enough amounts of beneficial dietary fats to impart any potential health benefits. Scollan and others (2001), for example, were able to greatly increase the amount of long chain n-3 PUFA in the phospholipid fraction of beef intramuscular fat by feeding linseed and fish oils. Phospholipids, however, only make up 2-8% of intramuscular lipid in beef (Campbell and Harrill 1971) and while it is an important factor in beef flavor (Mottram 1998) it is not present in great enough amounts to offer a significant contribution to overall dietary fat. In the same study, C20 and C22 fatty acids were not detected in the muscle lipid, while the amount of 18:3n-3 present in the intramuscular fat of the linseed-fish oil treatment cattle was 12 mg per 100 g of muscle. Considering the proposed optimal intake of 18:3n-3 is 2.2 g (Kolanowski and Laufenberg 2006), the cattle in this study have not yielded meat that, based on scientific evidence, helps a consumer to achieve the health benefits associated with intake of n-3 PUFA.

In contrast, a Dutch style fermented sausage, produced by Pelsler and others (2007) to contain flaxseed oil offers 4.33 g/100 g of product n-3 PUFA while maintaining a n-6:n-3 ratio of less than 1 (Jimenez-Colmenero 2007). In comparison to dietary or genetic approaches, processing techniques seem to be more suited to offer large

concentrations of nutritionally beneficial fatty acids to allow consumers to reasonably expect to see the health benefits associated with them. It is important to realize that even if PUFA:SFA, n-6:n-3, and MUFA:SFA ratios are optimum, products that present a disproportionately small or large percentage of total dietary fat energy would offer little benefit. It is also important that clinical trials be conducted to ensure that potentially beneficial products, developed based on generally accepted scientific data, are producing the desired effects in human subjects.

When working with processed meat products, such as ground beef, the issue of labeling must be addressed. According to the FSIS Food Standards and Labeling Policy Book (USDA 2005), ground beef has a maximum total fat percentage of 30 and may not contain added fat or organ meats. Any ground beef product containing these cannot be labeled as ground beef, chopped beef, or hamburger. Hamburger patties may contain soy products as long as the resulting combination product is not nutritionally inferior to ground beef. Any combination products found to be nutritionally inferior must be labeled as Imitation Hamburger, Imitation Ground Beef, Beef Patty or Ground Beef Patty Mix.

### **Summary of literature**

The growing amount of evidence that dietary fat intake can play a direct role in managing risk factors for certain cancers and cardiovascular diseases opens the doors for meat products, a significant source of fat in the western diet, to play a role as functional foods. Researchers have shown that fatty acid composition of meat can be altered to include more polyunsaturated fatty acids through dietary means, mainly grass feeding or supplementation with lipid supplements. Previous research also shows that processed meat products can be manufactured with replacement of animal fats with vegetable or marine oils to include beneficial fatty acids, such as monounsaturated fatty acids or long chain n-3 polyunsaturated fatty acids, at nutritionally significant levels. Careful design and

implementation of production strategies using one or both of the previously mentioned approaches can be used to offer meat products as functional foods. Design and formulation of functional meat products must also take into consideration the shelf life, sensory, texture, appearance issues presented throughout this text to ensure consumer acceptance.

It is important for the industry to innovate and incorporate new ideas to use in the production of functional foods that will maintain shelf life stability and sensory appeal. Rhoades and others (2005) formulated several lipid solution blends of beef tallow (BT) and high oleic safflower oil that were high in monounsaturated fatty acids (MUFA) and presented a color similar to that of beef intramuscular fat. Incorporation of this blend into muscle foods could provide a fat source that combines the look and consistency of animal fat with the ability to make alterations to the fatty acid profile by including various oils. The objective of this study is to determine if beef patties formulated with a blend of beef fat and vegetable oils will show an increase in unsaturated fatty acid content while maintaining desirable sensory attributes and adequate shelf life when compared to control beef patties.

## CHAPTER II

### MATERIALS AND METHODS

This section contains detailed descriptions of the procedures, processes, and equipment used to carry out this research study.

#### **Lipid manufacture**

Lipid blends were manufactured by weighing out portions of beef tallow (Proliant Frozen Beef Tallow, Proliant, Arkeny, IA) and either high oleic safflower oil (Butcher Boy High Oleic Safflower Oil, Columbus Foods, Chicago, IL), olive oil (Del Destino 100% Pure Olive Oil, Atalanta Corp. Elizabeth, NJ), or corn oil (Hill Country Fare 100% Pure Corn Oil, HEB, San Antonio, TX) to achieve a 57% beef tallow/43% edible oil mixture. A rosemary extract (Herbalox Type HT-25, Kalsec Inc., Kalamazoo, MI) was added at 0.3% directly to the oils to act as an antioxidant. Lipid blends were mixed in a bowl chopper (Seydelmann Model K-64, Maschinenfabrik Seydelmann KG, Stuttgart, Germany) with a 3 blade setup by chopping the beef tallow in the bowl chopper at 2000 rpm for 30 seconds before adding the edible oil and chopping at 4000 rpm for another 15 seconds. The mixture was then evacuated from the bowl chopper into a plastic container (Model 16PP, Cambro Manufacturing Company, Huntington Beach, CA). Samples were immediately taken for lipid oxidation and fat content analyses. Duplicate samples of each lipid blend, which were to be used for objective color, were poured into a glass petri dish, covered with saran wrap (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA) and stored at 6°C to solidify. The lipid blends were frozen at -10°C. Once the mixture was frozen it was removed from the container, vacuum packaged and stored at -10°C. The frozen lipid blend was hand cut into fist sized pieces and chopped for 15 seconds at 2000 rpm in the bowl chopper to a particle size of approximately 0.64 cm before being weighed out, placed in a 15 liter plastic bucket (Model RFS8PP, Cambro Manufacturing Company,

Huntington Beach, CA), covered with saran (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA), sealed with a plastic lid and stored at -10°C for 30 minutes until addition to the beef.

### **Raw material preparation**

Lean beef (Inside rounds, IMPS 169A, Denuded) and fat beef (Beef plates, IMPS 121) was received from a local processor and ground (Biro Model 10-56, Biro Mfg. Co. Marblehead, OH) separately through a 0.95 cm plate. The ground inside rounds and beef plates were mixed separately in a paddle mixer (Butcher Boy Model 150, Lasar MFG Inc., Los Angeles, CA) for 2 minutes and samples were taken for fat content and lipid oxidation analyses prior to patty formulation.

### **Control patty manufacture**

Control batches (CT 10% and CT 20%) of 13.6 kg were formulated to either 10% or 20%. The ground lean beef was added to the bowl chopper and chopped for 15 seconds at 2000 rpm before the addition of the ground fat beef. The mixture of lean and fat beef was chopped for another 15 seconds at 2000 rpm to achieve a particle definition of approximately 0.31 cm. The ground meat mixture was then evacuated from the bowl chopper into a plastic meat lug and placed into a commercial patty machine (Model 8/65, Hollymatic Corporation, Countryside, IL). Circular-shaped patties weighing approximately 113 g were produced.

### **Treatment patty manufacture**

Treatment batches of 13.6 kg were formulated by adding the high oleic safflower (SO), olive oil (OO), or corn oil (CO) lipid blends (97% fat) to the lean beef (6% fat) to obtain a final fat percentage of either 10% or 20% (Table 1). Ground lean was introduced



into the bowl chopper and chopped for 30 seconds at 2000 rpm before the addition of the appropriate chopped lipid blend. After another 30 seconds of chopping at 2000 rpm, the mixture was evacuated from the bowl chopper into a lug and placed into a patty machine (Model 8/65, Hollymatic Corporation, Countryside, IL). Patties weighing approximately 113g were produced.

### **Sample packaging**

Treatment and control patties were either boxed in a 43.2 cm wide x 25.4 cm long x 10.2 cm deep plastic lined cardboard patty boxes for frozen (-10°C) storage or overwrapped on 10S styrofoam trays (Sealed Air Corporation, Elmwood Park, NJ) with PVC film (Resinite RMF 61-HY stretch film, AEP Industries, Inc. Hackensack NJ) for refrigerated retail storage. Frozen patties were used for lipid oxidation, pH and objective color analyses on days 0, 7, 14, 28, 56, as well as cook yield, dimensional changes and shear force determination on day 28. Overwrapped refrigerated patties were used for lipid oxidation, pH and objective color analyses on days 0, 3, 6 and 9. 16 patties from each treatment were vacuum packaged in 17.8 cm X 25.4 cm vacuum pouches and frozen (-10°C) to be used for trained sensory panel analysis.

### **Refrigerated retail and frozen beef patty shelf life study**

Four overwrapped trays each containing two patties laid side by side, from each treatment were stored at 6°C in a cooler under fluorescent lights (Philips F40T12-CWT) at an intensity of 2150 Lux. Light intensity was measured using a light probe (Sper Model 850075 Sper Scientific, LTD Scottsdale, AZ) attached to an environmental quality meter (Sper Model 850071 Sper Scientific, LTD Scottsdale, AZ). The trays were stored for 3, 6, or 9 d before being removed for analyses. Each sample was used for objective color, lipid oxidation, and pH. Control and treatment patties were either placed in plastic-lined

cardboard boxes in and stored at  $-20^{\circ}\text{C}$  for 7, 14, 28, 42, or 56 d and then analyzed for lipid oxidation, objective color, and pH.

### **Lipid blend objective color**

Lipid blend samples, which were poured into a glass petri dish, covered with saran wrap (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA) and stored at  $6^{\circ}\text{C}$  to solidify were used to determine objective color. Color readings were taken in duplicate on each sample at the exposed surface using a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) with a 1.54 cm aperture, calibrated with white and black standards covered with saran wrap (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA). CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space values were calculated using illuminant A and a  $10^{\circ}$  observer.

### **pH and proximate composition**

The pH of raw and cooked beef patties was determined by inserting a pH probe attached to a pH meter (IQ Model IQ150 IQ Scientific Instruments, Inc. Reston VA) into patties from each treatment. Duplicate readings were taken on four samples from each treatment. The pH meter was calibrated with buffers 4.01 and 7.0.

Percent moisture and fat were determined using modified AOAC (2005) air-dry oven and soxhlet ether extraction methods, respectively (AOAC 2005). Powdered raw and cooked beef patty samples (~2.5 g) were placed in pre-weighed, previously dried paper thimbles (Whatman #2 filter paper) and the thimble plus sample weights were recorded. Samples were dried for 16 h at  $100^{\circ}\text{C}$ , cooled to room temperature in a desiccator, and the dried thimble plus sample weights recorded. Percent moisture was calculated as the difference between wet weight and dried sample weight divided by sample weight. Oven dried samples were then placed into a soxhlet apparatus, three

randomly selected thimbles per soxhlet, and extracted with petroleum ether for 12 hours. The thimbles were dried overnight to remove excess moisture, and percent fat was calculated as the difference between dried sample weight and extracted sample weight divided by sample weight. Percent protein was determined using a Leco FP-528 (Leco Corporation, St. Joseph, MI) nitrogen analyzer which vaporized powdered samples of 0.15 gram to release total nitrogen. Three samples per treatment were analyzed. Percent protein was calculated as 6.25 times the percent nitrogen (AOAC 2005).

### **Lipid oxidation**

Raw (refrigerated – 0, 3, 6 and 9 d at 6°C and frozen- 0, 7, 14, 28, 42 and 56 d at -20°C) patties and cooked beef patties (day 28 of frozen storage) were analyzed for lipid oxidation as determined by the thiobarbituric acid test of Tarladgis and others (1960) as modified by Rhee (1978). Two raw beef samples (60 g) per treatment taken from raw beef patties were blended with 90 ml of distilled water and 5 ml of antioxidant solution with 0.5% propyl gallate and 0.5% ethylenediamine tetraacetic acid. Thirty g blended samples were collected and combined with an additional 77.5 ml of distilled water and 2.5 ml of 4 N HCl in a Kjeldahl flask. The acidified sample was distilled and 50 ml of distillate collected. Following distillation, 5 ml of distillate was added to 5 ml of 0.02 M TBA reagent then heated in boiling water for 35 min to fully develop the color reaction. Absorbance was measured at 530 nm using an UV-visible spectrophotometer (Model Cary 300 Bio, Varian Instruments, Sugarland, TX). Results were reported as mg of malonaldehyde per kg of meat.

### **Beef patty objective color determinations**

Patties for the refrigerated retail shelf-life study were overwrapped and stored at 6°C under fluorescent lighting for 0, 3, 6, or 9 d. Color measurements were taken on the

surface of four patties from each treatment. The patty was divided into four quadrants and color measurements were taken from each quadrant. Color measurements were taken with a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) using a 1.54 cm aperture, calibrated with white and black standards covered with RMF-61HY film (Resinite RMF 61-HY stretch film, AEP Industries, Inc. Hackensack NJ). CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space values were calculated using illuminant A and a 10° observer.

Frozen raw patties were allowed to thaw for 12 hours at 6°C. Color measurements were taken on the surface of two patties from each treatment at four places on each patty. Color measurements were taken with a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) using a 1.54 cm aperture, calibrated with white and black standards. CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space values were calculated using illuminant A and a 10° observer.

Cooked beef patties were allowed to cool to 22°C and cut in half. Each patty was arranged so that the exposed internal surfaces of the patty were side by side. Color measurements were taken in 3 places on the internal surface of the 2 combined portions using a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) with a 1.54 cm aperture, calibrated with white and black standards. CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space values were calculated using illuminant C and a 10° observer.

### **Fatty acid composition**

Lipids were extracted from lipid blend, raw patty, and cooked patty samples from each treatment using the method of Folch and others (1957) and methylated by the method of Slover and Lanza (1979). Methylated lipids were analyzed with a Varian gas chromatograph (Model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA), equipped with a fused silica capillary column CP-Sil88 [100 m · 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas (flow

rate = 1.2 ml/min) (Smith and others 2002). After 32 min at 180°C, oven temperature was increased at 20°C/min to 225°C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270 and 300°C, respectively. Individual methylated lipids were quantified as g fatty acid/100 g of total FAME identified. Identities of FAME were established by comparison to authentic standards (GLC 96; Nu-Chek Prep, Inc, Elysian, MN, USA).

### **Allo-Kramer shear force determination and dimensional changes**

Ten beef patties from each treatment were measured for diameter and thickness at four places, equally spaced from one another, using a set of calipers. The patties were then cooked to 71°C according to AMSA (1995) and allowed to cool to approximately 22°C. Cook yields were determined using the formula (Final cook weight – Initial cook weight) \* 100. The patties were measured for diameter and thickness in four equally spaced places after cooking, using a set of calipers. Dimensional changes were reported as pre-cook measurements minus post-cook measurements. A 60 x 50 mm rectangular sample was excised from the center of each patty. The samples were weighed and shear values were recorded using an Instron Universal Testing Machine (Instron Corp., Canton, Mass., U.S.A.) equipped with a 10-blade Allo–Kramer shear compression cell using a 500-kg load cell with a load range of 200 kg and a crosshead speed of 200 mm/min. Shear values were reported as Newtons/gram (N/g).

### **Trained sensory panel analysis**

A trained descriptive attribute sensory panel was used to evaluate cooked beef patties for flavor, mouth feel, basic tastes, aftertastes and texture. Five panelists were selected and trained according to AMSA (1995) and Meilgaard and others (2007). Training was conducted prior to testing to familiarize the panelist with the attributes

cooked beef patties. Cooked beef samples were evaluated for texture (juiciness, sandy/gritty), flavor (cooked beef lean, cooked beef fat, oil, cardboard, painty, fishy, liver, browned, salt, sour, bitter, metallic, astringent), after mouth feel (oily, fat coating), and aftertastes (sour, bitter, browned, metallic, astringent, oily, fatty). All samples were scored using the 15 point Spectrum intensity scale (Meilgaard and others 2007) where 0 = absence of an attribute and 15 = extremely intense. Panelists evaluated 24 samples (8 samples per day for 3 days). Vacuum packaged beef patties were allowed to thaw for 12 hours at 6°C before being cooked to 71°C on Hamilton Beach Healthsmart grills according to AMSA (1995), cut into 8 wedges and served to the panelists in warmed custard dishes. Each panelist received two wedges per sample.

### **Statistical analysis**

Proximate composition, shear force, cook yield, dimensional changes, objective color, lipid oxidation and fatty acid analyses were statistically analyzed as a completely randomized block design using the Mixed Model procedure of the Statistical Analysis System (Version 9.1, SAS Institute, Inc., Cary, NC). The model for all dependant variables included the fixed effect of lipid blend treatment and the block effect of replication. Repeated measures data (objective color and lipid oxidation) also included the fixed effect of storage day and the interaction between lipid blend treatment and storage day. Storage day was defined as a repeated effect. Differences between treatment means were separated with Tukey's studentized range test for significant main effects at  $P < 0.05$ . Trained sensory panel data were statistically analyzed using the Mixed Model of the Statistical Analysis System (Version 9.1, SAS Institute, Inc., Cary, NC). The model for all dependant variables included the block effect of replication and the fixed effect of lipid blend treatment. All variables were analyzed for a significant interaction between lipid blend treatment and panelist before being pooled across all

panelists. Differences among treatment means were identified using Tukey's studentized range test for significant main effects at  $P < 0.05$ .

## CHAPTER III

### RESULTS AND DISCUSSION

#### **Introduction**

Increased consumer awareness of the importance of dietary fat to human health has led to an interest in finding ways to manipulate the fatty acid composition of meat products, which are seen as a significant source of fat in the diet. The status of red meat as a nutritious staple of western diets, rich in high quality protein as well as micronutrients, has been overshadowed by concerns about the amount of saturated fatty acids (SFA) in red meat products, as well as the lack of essential polyunsaturated fatty acids (PUFA), especially long chain n-3 PUFA. The connection between these factors and incidences of certain cancers and cardiovascular diseases is well established (Leaf and others 2003; Calder 2004). As a result, work has been conducted on novel approaches to altering the fatty acid profile of red meat products to more closely resemble recommended nutrient intake goals.

Previous work on preslaughter dietary approaches to alter the fatty acid profile of red meats includes dietary supplementation of various oils to increase deposition of unsaturated fatty acids in the intramuscular and subcutaneous fat of cattle and pigs (Nuernberg and others 2005; Vatansever and others 2000). While research has shown that increasing the amount of unsaturated fats in the diet does alter the fatty acid profile of the animals, it also increases the rate of oxidation of raw materials from these animals (Kouba and others 2003; Vatansever and others 2000).

Another, perhaps more efficient and versatile approach, involves incorporation of vegetable and marine oils into processed meat products to replace animal fat. This approach has been used to make frankfurters (Vural and others 2004), sausages (Pelser and others 2007; Bloukas and others 1997) and beef patties (Shiota and others 1995).



While reducing saturated fatty acid content has advantages from a nutritional standpoint, it can present problems such as reduced shelf life and altered appearance or texture (Jimenez-Colmenero 2007).

Rhoades and others (2005) formulated several lipid solution blends of beef tallow (BT) and high oleic safflower oil that were high in monounsaturated fatty acids (MUFA) and possessed a color similar to that of beef subcutaneous fat. A mixture such as this could be beneficial in an effort to produce a product that is higher in unsaturated fatty acids but retains a similar appearance and texture to that of a product manufactured with beef fat.

We hypothesized that beef patties formulated with a blend of beef fat and vegetable oils would show an increase in unsaturated fatty acid content while maintaining desirable sensory attributes and adequate shelf life when compared to control beef patties.

## **Materials and methods**

### **Lipid manufacture**

Lipid blends were manufactured by weighing out portions of beef tallow (BT; Proliant Frozen Beef Tallow, Proliant, Arkeny, IA) and either high oleic safflower oil (SO; Butcher Boy High Oleic Safflower Oil, Columbus Foods, Chicago, IL), olive oil (OO; Del Destino 100% Pure Olive Oil, Atalanta Corp. Elizabeth, NJ), or corn oil (CO; Hill Country Fare 100% Pure Corn Oil, HEB, San Antonio, TX) to achieve a 57% beef tallow/43% edible oil mixture (Table 1). A rosemary extract (Herbalox Type HT-25, Kalsec Inc., Kalamazoo, MI) was added at 0.3% directly to the oils to act as an antioxidant. Lipid blends were mixed in a bowl chopper (Seydelmann Model K-64, Maschinenfabrik Seydelmann KG, Stuttgart, Germany) with a 3 blade setup by chopping the beef tallow in the bowl chopper at 2000 rpm for 30 seconds before adding the edible oil and chopping at

2000 rpm for another 15 seconds. The mixture was then evacuated from the bowl chopper into a plastic container and samples were immediately taken for lipid oxidation and fat content analyses. Duplicate samples of each lipid blend, which were to be used for objective color, were poured into a glass petri dish, covered with saran wrap (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA) and stored at 6°C to solidify. The lipid blends were frozen at -10°C. Once the mixture was frozen it was removed from the container, vacuum packaged and stored at -10°C. The frozen lipid blend was hand cut into fist sized pieces and chopped for 15 seconds at 2000 rpm in the bowl chopper to a particle size of approximately 0.64 cm before being weighed out, placed in a 15 liter plastic bucket (Model RFS8PP, Cambro Manufacturing Company, Huntington Beach, CA), covered with saran (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA), sealed with a plastic lid and stored at -10°C for 30 minutes until addition to the beef according to formulations (Table 1).

### **Raw material preparation**

Lean beef (Inside rounds, IMPS 169A, Denuded) and fat beef (Beef plates, IMPS 121) was received from a local processor and ground (Biro Model 10-56, Biro Mfg. Co. Marblehead, OH) separately through a 0.95 cm plate. The ground inside rounds and beef plates were mixed separately in a paddle mixer (Butcher Boy Model 150, Lasar MFG Inc., Los Angeles, CA) for 2 minutes and samples were taken for fat content and lipid oxidation analyses prior to patty formulation.

### **Control patty manufacture**

Batches (13.6 kg) were formulated at either 10 or 20% fat for control (CT) patty manufacture (Table 1). The ground lean beef was added to the bowl chopper and chopped for 15 seconds at 2000 rpm before the addition of the ground fat beef. The

Table 1. Formulation weights (kg) for manufacture of beef patties containing a blend of beef tallow (57%) and mono- or poly unsaturated oils (43%)

	CT 10 10% <sup>a</sup>	SO 10 10% <sup>b</sup>	OO 10 10% <sup>b</sup>	CO 10 10% <sup>b</sup>	CT 20 20% <sup>a</sup>	SO 20 20% <sup>b</sup>	OO 20 20% <sup>b</sup>	CO 20 20% <sup>b</sup>
Beef Patties								
<b>Meat Block</b>								
Beef inside rounds- 94/6 <sup>c</sup>	12.07	12.97	12.97	12.97	8.30	11.48	11.48	11.48
Beef Plates-58/42 <sup>c</sup>	1.54				5.31			
<b>Lipid Blends</b>								
Beef tallow		0.36	0.36	0.36		1.22	1.22	1.22
High Oleic Safflower Oil - 95.51% <sup>d</sup>		0.27				0.92		
Olive Oil - 95.69% <sup>d</sup>			0.27				0.92	
Corn Oil - 96.01% <sup>d</sup>				0.27				0.92
Lipid Blend Total		0.64	0.64	0.64		2.13	2.13	2.13
<b>Total Weight (kg)</b>	13.61	13.61	13.61	13.61	13.61	13.61	13.61	13.61

<sup>a</sup>Fat levels represent a mixture of beef fat present in the beef lean trim (6%) and the beef plates (42%) formulated to achieve the desired 10% and 20% fat levels

<sup>b</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels

<sup>c</sup>Lean/Fat ratio of selected materials

<sup>d</sup>Total fat percentage by weight of lipid blends

mixture of lean and fat beef was chopped for another 15 seconds at 2000 rpm to achieve a particle size of approximately 0.31 cm. The ground meat mixture was then evacuated from the bowl chopper into a plastic meat lug and placed into a commercial patty machine (Model 8/65, Hollymatic Corporation, Countryside, IL). Circular-shaped patties weighing approximately 113 g were produced.

### **Treatment patty manufacture**

Batches (13.6 kg) were formulated by adding either high oleic safflower (SO), olive oil (OO), or corn oil (CO) lipid blends (97% fat) to the lean beef (6% fat) to obtain a final raw patty fat percentage of either 10 or 20% (Table 1). Ground lean (-1.1°C) was introduced into the bowl chopper and chopped for 30 seconds at 2000 rpm before the addition of the appropriate type and amount of chopped lipid blend. Final patty particle size (0.31 cm) was attained after another 30 seconds of chopping at 2000 rpm. The mixture was evacuated from the bowl chopper into a lug and placed into a patty machine (Model 8/65, Hollymatic Corporation, Countryside, IL). Patties weighing approximately 113g were produced.

### **Sample packaging**

Treatment and control patties were either boxed in a 43.2 cm wide x 25.4 cm long x 10.2 cm deep plastic lined cardboard patty boxes for frozen (-10°C) storage or overwrapped on 10S styrofoam trays (Sealed Air Corporation, Elmwood Park, NJ) with PVC film (Resinite RMF 61-HY stretch film, AEP Industries, Inc. Hackensack NJ) for refrigerated retail storage. Frozen patties were used for lipid oxidation, pH and objective color analyses on days 0, 7, 14, 28, 56, as well as cook yield, dimensional changes and

shear force determination on day 28. Overwrapped refrigerated patties were used for lipid oxidation, pH and objective color analyses on days 0, 3, 6 and 9. 16 patties from each treatment were vacuum packaged in 17.8 cm X 25.4 cm vacuum pouches and frozen (-10°C) to be used for trained sensory panel analysis.

### **Refrigerated retail and frozen beef patty shelf life study**

Four patties (two stacks of two patties each arranged side by side) from each control and treatment patty formulation were placed on 10S Styrofoam trays overwrapped with oxygen permeable polyvinyl chloride (PVC) film and stored at 6°C under fluorescent lights (Philips F40T12-CWT) at an intensity of 2150 Lux. The trays were stored for 3, 6, or 9 d before being removed for analyses. Each sample (2 patties) was used for objective color, lipid oxidation, and pH. Control and treatment patties were either placed in plastic-lined cardboard boxes in and stored at -20°C for 7, 14, 28, 42, or 56 d and then analyzed for lipid oxidation, objective color, and pH.

### **Lipid blend objective color**

Lipid blend samples, which were poured into a glass petri dish, covered with saran wrap (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA) and stored at 6°C to solidify were used to determine objective color. Color reading were taken in duplicate on each sample at the exposed surface using a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) with a 1.54 cm aperture, calibrated with white and black standards covered with saran wrap (Reynolds Foodservice Film, Reynolds Food Packaging, Richmond, VA). CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space values were calculated using illuminant A and a 10° observer.

### **pH and proximate composition**

The pH of raw and cooked beef patties was determined by inserting a pH probe attached to a pH meter (IQ Model IQ150 IQ Scientific Instruments, Inc. Reston VA) into patties from each treatment. Duplicate readings were taken on four samples from each treatment. The pH meter was calibrated with buffers 4.01 and 7.0.

Percent moisture and fat were determined using modified AOAC (2005) air-dry oven and soxhlet ether extraction methods, respectively (AOAC 2005). Percent protein was determined using a Leco FP-528 (Leco Corporation, St. Joseph, MI) nitrogen analyzer which vaporized powdered samples of 0.15 gram to release total nitrogen. Percent protein was calculated as 6.25 times the percent nitrogen.

### **Lipid oxidation**

Lipid oxidation was determined by the thiobarbituric acid test of Tarladgis and others (1960) as modified by Rhee (1978). Absorbance was measured at 530 nm using an UV-visible spectrophotometer (Model Cary 300 Bio, Varian Instruments, Sugarland, TX). Results were reported as mg of malonaldehyde per kilogram of meat.

### **Beef patty objective color determinations**

Color measurements were taken on the surface of raw patties from each treatment using a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) with a 1.54 cm aperture, calibrated with white and black standards covered by a piece of RMF-61HY overwrap film (Resinite RMF 61-HY stretch film, AEP Industries, Inc. Hackensack NJ). CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space values were calculated using illuminant A and a 10° observer.

Cooked beef patties were allowed to cool to 22°C and cut in half. Each patty was folded in half so that the exposed internal surfaces of the patty were side by side. Color measurements were taken in 3 places on the internal surface of the 2 combined portions using a Hunter Miniscan XE (Model 45/O-L, Hunter Associates Laboratory, Inc. Reston VA) with a 1.54 cm aperture, calibrated with white and black standards. CIE  $L^*$ ,  $a^*$ , and  $b^*$  color space values were calculated using illuminant C and a 10° observer.

### **Fatty acid composition**

Lipids were extracted using the method of Folch and others (1957) and methylated by the method of Slover and Lanza (1979). Methylated lipids were analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA), equipped with a fused silica capillary column CP-Sil88 [100 m · 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas (flow rate = 1.2 ml/min) (Smith et al., 2002). After 32 min at 180°C, oven temperature was increased at 20°C/min to 225°C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270 and 300°C, respectively. Individual methylated lipids were quantified as g fatty acid/100 g of total FAME identified. Identities of FAME were established by comparison to authentic standards (GLC 96; Nu-Chek Prep, Inc, Elysian, MN, USA).

### **Allo-Kramer shear force determination and dimensional changes**

Ten beef patties from each treatment were measured for diameter and thickness at four places, equally spaced from one another, using a set of calipers. The patties were then cooked to 71°C according to AMSA (1995) and allowed to cool to

approximately 22°C. The patties were measured for diameter and thickness in four equally spaced places after cooking, using a set of calipers. Dimensional changes were reported as pre-cook measurements minus post-cook measurements. A 60 x 50 mm rectangular sample was excised from the center of each patty. The samples were weighed and shear values were recorded using an Instron Universal Testing Machine (Instron Corp., Canton, Mass., U.S.A.) equipped with a 10-blade Allo–Kramer shear compression cell using a 500-kg load cell with a load range of 200 kg and a crosshead speed of 200 mm/min. Shear values were reported as Newtons/gram (N/g).

### **Trained sensory panel analysis**

A trained descriptive attribute sensory panel was used to evaluate cooked beef patties for flavor, mouth feel, basic tastes, aftertastes and texture. Five panelists were selected and trained according to AMSA (1995) and Meilgaard and others (2007). Training was conducted prior to testing to familiarize the panelist with the attributes cooked beef patties. Cooked control and treatment patties were evaluated for texture (juiciness, sandy/gritty), flavor (cooked beef lean, cooked beef fat, oil, cardboard, painty, fishy, liver, browned, salt, sour, bitter, metallic, astringent), after mouth feel (oily, fat coating), and aftertastes(sour, bitter, browned, metallic, astringent, oily, fatty). All samples were scored using the 15 point Spectrum intensity scale (Meilgaard and others 2007) where 0 = absence of an attribute and 15 = extremely intense. Panelists evaluated 24 samples (8 samples per day for 3 days). Beef patties were cooked to 71°C on Hamilton Beach Healthsmart grills according to AMSA (1995), cut into 8 wedges and served to the panelists in warmed custard dishes. Each panelist received two wedges per sample.



## **Statistical analysis**

Proximate composition, shear force, cook yield, dimensional changes, objective color, lipid oxidation and fatty acid analyses were statistically analyzed as a completely randomized block design using the Mixed Model procedure of the Statistical Analysis System (Version 9.1, SAS Institute, Inc., Cary, NC). The model for all dependant variables included the fixed effect of lipid blend treatment and the block effect of replication. Repeated measures data (objective color and lipid oxidation) also included the fixed effect of storage day and the interaction between lipid blend treatment and storage day. Storage day was defined as a repeated effect. Differences between treatment means were separated with Tukey's studentized range test for significant main effects at  $P < 0.05$ . Trained sensory panel data was statistically analyzed using the Mixed Model of the Statistical Analysis System (Version 9.1, SAS Institute, Inc., Cary, NC). The model for all dependant variables included the block effect of replication and the fixed effect of lipid blend treatment. All variables were analyzed for a significant interaction between lipid blend treatment and panelist before being pooled across all panelists. Differences among treatment means were identified using Tukey's studentized range test for significant main effects at  $P < 0.05$ .

## **Results and discussion**

### **Objective color of lipid blends**

Means for objective color of lipid blends (BT/oil) are presented in Table 2.  $L^*$  values ranged from 77.02 to 81.52 with BT/OO being the highest, BT/CO being intermediate ( $P$  and BT/HOSO being the lowest in whiteness values. Redness was not affected by the type of lipid blend ( $P > 0.05$ ). BT/OO was highest ( $P \leq 0.05$ ) for

yellowness, while BT/HOSO blends were intermediate and BT/CO blends were the lowest.

### **Proximate composition**

Main effect least squares means for fat, moisture and protein percentages are presented in Table 3. Data showed that raw patties closely approximate their 10% and 20% formulations. Both the 10 and 20% controls were lower in fat percentage than the other treatments. As expected, all 20% fat treatments were higher in fat percentage and lower in protein and moisture percentage than the 10% fat treatments.

For cooked patties CT 20 was higher for cooked fat percentage than the other 20% treatment patties. This is most likely due to the higher melting point of the saturated fatty acids present in the control patties. All 20% fat cooked patties were higher in fat percentage than the 10% fat patties. For all treatments, the 10% cooked patties were higher in protein and moisture percentage than the 20% patties.

### **Comparison of TBA values during refrigerated storage**

The two way interaction of lipid blend treatment x storage day was significant for TBA values of the control and treatment patties (Table 4). TBA values for all treatments increased ( $P < 0.05$ ) with storage time. TBA values on d 0 were not affected by lipid blend treatment. At d 3 CT 20 had a higher TBA value than all other treatments, most likely due to the absence of the rosemary extract present in the treatment patties and its results suggest that the patties formulated with lipid blend treatments did not oxidize any faster than the controls. Dzudie and others (2004) saw similar results for beef patties

Table 2. Least squares means for L\*, a\*, and b\* values of lipid blends formulated with beef tallow (57%) and mono- or polyunsaturated oils (43%)

Color <sup>d</sup>	BT/High Oleic Safflower	BT/Olive Oil	BT/Corn Oil
L*	77.02 <sup>c</sup> ± 0.40 <sup>e</sup>	81.52 <sup>a</sup> ± 0.46	79.57 <sup>b</sup> ± 0.40
a*	1.91 ± 0.03	1.81 ± 0.03	1.82 ± 0.03
b*	18.98 <sup>b</sup> ± 0.20	19.91 <sup>a</sup> ± 0.24	18.19 <sup>c</sup> ± 0.20

<sup>a-c</sup>Means in a row with different superscripts are significantly different (P < 0.05)

<sup>d</sup>L\* = lightness/whiteness; a\* = redness; b\* = yellowness

<sup>e</sup>Standard deviation

Table 3. Main effect means (fat level and lipid blend treatment) for proximate composition of raw and cooked beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%)

	CT		SO		OO		CO		SEM <sup>f</sup>
	Fat level (%) <sup>e</sup>								
	10	20	10	20	10	20	10	20	
Raw									
Fat %	10.41 <sup>d</sup>	19.29 <sup>b</sup>	12.04 <sup>c</sup>	21.01 <sup>a</sup>	11.35 <sup>cd</sup>	20.66 <sup>ab</sup>	10.09 <sup>d</sup>	20.89 <sup>a</sup>	0.33
Protein %	21.30 <sup>a</sup>	19.81 <sup>bc</sup>	20.66 <sup>ab</sup>	18.98 <sup>cd</sup>	21.00 <sup>a</sup>	18.68 <sup>d</sup>	21.13 <sup>a</sup>	18.89 <sup>cd</sup>	0.23
Moisture %	68.84 <sup>ab</sup>	62.41 <sup>c</sup>	67.77 <sup>b</sup>	61.00 <sup>d</sup>	68.74 <sup>ab</sup>	61.61 <sup>cd</sup>	69.36 <sup>a</sup>	61.24 <sup>d</sup>	0.24
Cooked									
Fat %	15.50 <sup>d</sup>	26.47 <sup>a</sup>	15.08 <sup>d</sup>	21.59 <sup>b</sup>	14.93 <sup>d</sup>	18.79 <sup>c</sup>	14.30 <sup>d</sup>	19.59 <sup>bc</sup>	0.94
Protein %	29.11 <sup>a</sup>	26.16 <sup>bc</sup>	27.61 <sup>ab</sup>	24.39 <sup>d</sup>	28.13 <sup>a</sup>	25.53 <sup>cd</sup>	28.38 <sup>a</sup>	24.59 <sup>cd</sup>	0.35
Moisture %	56.89 <sup>ab</sup>	48.89 <sup>e</sup>	56.72 <sup>abc</sup>	53.80 <sup>d</sup>	58.55 <sup>a</sup>	55.63 <sup>bcd</sup>	57.31 <sup>ab</sup>	54.78 <sup>cd</sup>	0.47

<sup>a-d</sup>Means in a column with different superscripts are significantly different ( $P < 0.05$ ).

<sup>e</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% total raw product fat levels. Control contained no lipid blend treatment.

<sup>f</sup>SEM = Standard error of the mean.

Table 4. Two-way interaction (lipid blend treatment x storage day) least squares means for TBA values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at 0, 3, 6, 9 days of storage<sup>g</sup>

Treatment	Fat Level (%) <sup>e</sup>	Day				SEM <sup>f</sup>
		0	3	6	9	
CT	10	0.23 <sup>w</sup>	1.55 <sup>x</sup>	2.39 <sup>abcx</sup>	4.38 <sup>bcy</sup>	0.23
	20	0.35 <sup>w</sup>	2.05 <sup>x</sup>	2.95 <sup>abcx</sup>	4.42 <sup>bcy</sup>	0.23
SO	10	0.34 <sup>w</sup>	1.35 <sup>w</sup>	1.90 <sup>cw</sup>	3.87 <sup>cx</sup>	0.23
	20	0.39 <sup>w</sup>	1.11 <sup>wx</sup>	2.11 <sup>cx</sup>	3.97 <sup>cy</sup>	0.23
OO	10	0.44 <sup>w</sup>	1.48 <sup>w</sup>	2.90 <sup>abcx</sup>	6.01 <sup>ay</sup>	0.23
	20	0.45 <sup>w</sup>	1.43 <sup>w</sup>	3.76 <sup>abx</sup>	6.77 <sup>ay</sup>	0.23
CO	10	0.46 <sup>w</sup>	1.44 <sup>w</sup>	2.64 <sup>bcx</sup>	5.66 <sup>aby</sup>	0.23
	20	0.41 <sup>w</sup>	0.94 <sup>wx</sup>	2.14 <sup>cx</sup>	5.54 <sup>aby</sup>	0.23

<sup>a-d</sup>Means with the same letter within a column are not significantly different ( $P < 0.05$ )

<sup>w-z</sup>Means with the same letter within a row are not significantly different ( $P < 0.05$ )

<sup>e</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels.

<sup>f</sup>SEM = Standard error of the mean.

<sup>g</sup>Refrigerated (6°C) patties were stored in PVC overwrapped trays under fluorescent lighting.

formulated with 20% maize oil instead of beef fat, which did not exhibit higher TBA values than those with animal fats. The authors concluded that tocopherol, a free radical scavenger native to beef tissue, acted as a natural antioxidant. The inclusion of a rosemary antioxidant along with the presence of tocopherol is likely why TBA values of lipid blend treatment patties were not observed to be higher than the control patties in this study.

### **Comparison of TBA values during frozen storage**

The two way interaction between lipid blend treatment and storage day was significant at  $P \leq 0.05$ . Least square means of TBA values for the two way interaction of lipid blend treatment x storage day are presented in Table 5. TBA values at d 0 were not affected by lipid blend treatment. Control patties and those containing high oleic safflower were not affected by storage day. Patties formulated with olive oil and corn oil increased in TBA values then decreased as storage time increased. A similar effect was seen by Nassu and others (2003) while using a natural rosemary antioxidant, similar to the one used in this study, to determine the oxidative stability of goat meat sausage. The authors attributed the reduction in TBA values to reactions of malonaldehyde, the compound used to measure TBA values, with proteins. TBA values ranged from 0.16 to 0.73 for all patty treatments across all storage days. None of the treatments reached the threshold TBA value of 1 over the 56 d of frozen storage. The oxidative stability over time of with patties high in unsaturated oils can be attributed to the antioxidant effect of the rosemary extract (St. Angelo and others 1990; Rojas and Brewer 2007), as well as its synergistic action with the tocopherols present in the treatment oils and beef raw materials (Wada and Fang 1992; Hras and others 2000).

Table 5. Two-way interaction (lipid blend treatment x storage day) least squares means for TBA values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at days 0, 7, 14, 28, 42, and 56 of storage<sup>g</sup>

Treatment	Fat level (%) <sup>e</sup>	Day						SEM <sup>f</sup>
		0	7	14	28	42	56	
CT	10	0.23	0.34 <sup>d</sup>	0.38	0.48 <sup>ab</sup>	0.41 <sup>ab</sup>	0.56 <sup>ab</sup>	0.06
	20	0.35	0.68 <sup>a</sup>	0.44	0.64 <sup>a</sup>	0.60 <sup>a</sup>	0.73 <sup>a</sup>	0.06
SO	10	0.34	0.54 <sup>ab</sup>	0.26	0.33 <sup>ab</sup>	0.34 <sup>ab</sup>	0.45 <sup>ab</sup>	0.06
	20	0.39	0.50 <sup>ab</sup>	0.18	0.29 <sup>ab</sup>	0.27 <sup>ab</sup>	0.41 <sup>ab</sup>	0.06
OO	10	0.44 <sup>wx</sup>	0.71 <sup>a,w</sup>	0.31 <sup>x</sup>	0.37 <sup>ab,wx</sup>	0.32 <sup>ab,x</sup>	0.41 <sup>ab,wx</sup>	0.06
	20	0.45 <sup>wx</sup>	0.68 <sup>ab,w</sup>	0.26 <sup>x</sup>	0.34 <sup>ab,wx</sup>	0.28 <sup>ab,x</sup>	0.42 <sup>ab,wx</sup>	0.06
CO	10	0.46 <sup>wx</sup>	0.62 <sup>ab,w</sup>	0.23 <sup>x</sup>	0.35 <sup>ab,wx</sup>	0.21 <sup>b,x</sup>	0.30 <sup>b,wx</sup>	0.06
	20	0.41 <sup>wx</sup>	0.52 <sup>ab,w</sup>	0.20 <sup>wx</sup>	0.19 <sup>b,x</sup>	0.16 <sup>b,x</sup>	0.25 <sup>b,wx</sup>	0.06

<sup>a-b</sup>Means with the same letter within a column are not significantly different ( $P < 0.05$ )

<sup>w-x</sup>Means with the same letter within a row are not significantly different ( $P < 0.05$ )

<sup>e</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels.

<sup>f</sup>SEM = Standard error of the mean.

<sup>g</sup>Frozen (-20°C) patties stored in plastic-lined cardboard boxes

### Refrigerated raw patty color determination

CIE  $L^*$ ,  $a^*$  and  $b^*$  values for refrigerated treatment and control patties are presented in Table 6.  $L^*$  (Lightness) values were not affected by lipid blend treatment ( $P > 0.05$ ), but all treatments excluding SO 20 and CO 10, became darker ( $P < 0.05$ ) between d 0 and d 6. SO 10 and 20 displayed the most redness ( $a^*$ ) with values of 30.83 and 30.15, respectively, on day 0. All treatments decreased ( $P < 0.05$ ) in redness by d 3 and they decreased ( $P < 0.05$ ) again by d 6. SO 10 was higher in yellowness ( $b^*$ ) than all other treatments except for SO 20 at d 0. Pelsler and others (2007) saw a similar effect in fermented sausages from using canola and flaxseed oils, which have a slightly yellow appearance. This is most likely not the cause in this study, as objective color determination showed the beef tallow/olive oil mixture to be more yellow ( $P < 0.05$ ) than the beef tallow/high oleic safflower oil mixture (Table 2), and the high oleic safflower oil treated patties were not significantly higher for  $b^*$  values than any other treatment on any subsequent day. Yellowness decreased ( $P < 0.05$ ) for all treatments between d 0 and d 3, but did not decrease any further.

### Frozen raw patty color determination

CIE  $L^*$ ,  $a^*$  and  $b^*$  values of frozen treatment and control patties are presented in Table 7. Lipid blend treatment had no effect on lightness ( $L^*$ ) values of frozen patties. Storage day did affect ( $P < 0.05$ ) lightness values, with the highest values for all treatments displayed at d 14. Redness values did not show any differences between treatments on any day except d 7, when SO 10 displayed the highest value (27.30). Redness values for all treatments tended to decrease ( $P < 0.05$ ) as storage time increased. Yellowness values were highest ( $P < 0.05$ ) for SO 10 (33.24) compared to all



Table 6. Two way interaction (lipid blend treatment x storage day) least squares means of L\*, a\*, and b\* values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at days 0, 3, 6, and 9 of refrigerated storage<sup>g</sup>

	CT		SO		OO		CO		SEM
	Fat level (%) <sup>e</sup>								
	10	20	10	20	10	20	10	20	
Day 0									
L*	53.15 <sup>a</sup>	47.52 <sup>a</sup>	48.34 <sup>a</sup>	47.38	48.69 <sup>a</sup>	45.21 <sup>a</sup>	48.70	47.11 <sup>a</sup>	1.80
a*	27.27 <sup>ax</sup>	28.51 <sup>awx</sup>	30.83 <sup>aw</sup>	30.15 <sup>awx</sup>	29.42 <sup>awx</sup>	29.12 <sup>awx</sup>	29.32 <sup>awx</sup>	27.72 <sup>ax</sup>	0.52
b*	22.24 <sup>ax</sup>	22.95 <sup>ax</sup>	28.75 <sup>aw</sup>	24.56 <sup>awx</sup>	23.88 <sup>ax</sup>	23.84 <sup>ax</sup>	23.47 <sup>ax</sup>	23.08 <sup>ax</sup>	0.83
Day 3									
L*	46.95 <sup>b</sup>	47.23 <sup>a</sup>	45.62 <sup>ab</sup>	46.80	44.68 <sup>ab</sup>	43.58 <sup>a</sup>	46.85	45.80 <sup>a</sup>	1.80
a*	13.12 <sup>bwx</sup>	12.34 <sup>bx</sup>	13.28 <sup>bwx</sup>	14.86 <sup>bwx</sup>	12.30 <sup>bx</sup>	12.91 <sup>bwx</sup>	14.03 <sup>bwx</sup>	15.37 <sup>bw</sup>	0.52
b*	14.44 <sup>b</sup>	14.69 <sup>b</sup>	14.42 <sup>b</sup>	15.73 <sup>b</sup>	14.07 <sup>b</sup>	14.10 <sup>b</sup>	15.09 <sup>b</sup>	15.87 <sup>b</sup>	0.83
Day 6									
L*	41.60 <sup>c</sup>	41.98 <sup>b</sup>	40.58 <sup>c</sup>	44.85	40.04 <sup>b</sup>	37.82 <sup>b</sup>	44.71	40.86 <sup>b</sup>	1.80
a*	6.76 <sup>c</sup>	7.84 <sup>c</sup>	6.94 <sup>c</sup>	7.20 <sup>c</sup>	6.46 <sup>c</sup>	6.31 <sup>c</sup>	6.56 <sup>c</sup>	6.88 <sup>c</sup>	0.52
b*	13.68 <sup>b</sup>	14.46 <sup>b</sup>	13.61 <sup>b</sup>	14.89 <sup>b</sup>	13.68 <sup>b</sup>	13.10 <sup>b</sup>	14.26 <sup>b</sup>	14.41 <sup>b</sup>	0.83
Day 9									
L*	42.77 <sup>bc</sup>	42.99 <sup>b</sup>	42.75 <sup>bc</sup>	46.67	41.56 <sup>b</sup>	40.63 <sup>ab</sup>	46.03	43.73 <sup>ab</sup>	1.80
a*	8.04 <sup>cx</sup>	11.62 <sup>bw</sup>	7.86 <sup>cx</sup>	7.39 <sup>cx</sup>	6.70 <sup>cx</sup>	7.71 <sup>cx</sup>	7.53 <sup>cx</sup>	7.09 <sup>cx</sup>	0.52
b*	14.47 <sup>b</sup>	15.47 <sup>b</sup>	14.22 <sup>b</sup>	15.88 <sup>b</sup>	14.17 <sup>b</sup>	14.38 <sup>b</sup>	15.32 <sup>b</sup>	15.54 <sup>b</sup>	0.83

<sup>a-d</sup>Means with the same letter within a column are not significantly different (P < 0.05)

<sup>w-z</sup>Means with the same letter within a row are not significantly different (P < 0.05)

<sup>e</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels

<sup>f</sup>SEM = Standard error of the mean.

<sup>g</sup>Refrigerated (6°C) patties were stored in PVC overwrapped trays under fluorescent lighting.

Table 7. Two way interaction (lipid blend treatment x storage day) least squares means of L\*, a\*, and b\* values of raw beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%) at days 0, 7, 14, 28, 42, and 56 of frozen storage<sup>g</sup>

		CT		SO		OO		CO		SEM
		Fat level (%) <sup>e</sup>								
		10	20	10	20	10	20	10	20	
Day 0	L*	51.46 <sup>a</sup>	48.59 <sup>ab</sup>	49.36 <sup>ab</sup>	48.34 <sup>b</sup>	47.57 <sup>ab</sup>	45.05 <sup>b</sup>	50.17 <sup>ab</sup>	46.10 <sup>b</sup>	1.34
	a*	28.35 <sup>a</sup>	28.58 <sup>a</sup>	30.92 <sup>a</sup>	30.32 <sup>a</sup>	29.12 <sup>a</sup>	29.11 <sup>a</sup>	28.91 <sup>a</sup>	27.54 <sup>a</sup>	0.69
	b*	22.86 <sup>x</sup>	23.14 <sup>x</sup>	33.24 <sup>aw</sup>	24.81 <sup>x</sup>	23.67 <sup>x</sup>	23.70 <sup>x</sup>	23.18 <sup>x</sup>	23.10 <sup>x</sup>	1.36
Day 7	L*	46.11 <sup>ab</sup>	44.37 <sup>ab</sup>	43.10 <sup>bc</sup>	44.02 <sup>b</sup>	43.27 <sup>b</sup>	41.94 <sup>b</sup>	42.98 <sup>c</sup>	44.18 <sup>b</sup>	1.34
	a*	25.09 <sup>abwx</sup>	24.70 <sup>abwx</sup>	27.30 <sup>aw</sup>	24.21 <sup>abwx</sup>	26.23 <sup>abwx</sup>	23.89 <sup>abwx</sup>	26.13 <sup>abwx</sup>	22.98 <sup>bx</sup>	0.69
	b*	19.86	19.70	21.30 <sup>b</sup>	19.68	20.96	19.19	20.31	23.10	1.36
Day 14	L*	52.02 <sup>a</sup>	55.14 <sup>a</sup>	51.69 <sup>a</sup>	55.81 <sup>a</sup>	51.28 <sup>a</sup>	53.49 <sup>a</sup>	53.75 <sup>a</sup>	56.75 <sup>a</sup>	1.34
	a*	22.41 <sup>b</sup>	21.51 <sup>c</sup>	23.23 <sup>cd</sup>	22.09 <sup>bc</sup>	22.08 <sup>c</sup>	22.59 <sup>bc</sup>	22.65 <sup>c</sup>	22.13 <sup>b</sup>	0.69
	b*	17.28	17.81	18.10 <sup>b</sup>	19.11	17.22	18.97	18.41	20.03	1.36
Day 28	L*	45.32 <sup>ab</sup>	45.11 <sup>b</sup>	42.49 <sup>bc</sup>	45.36 <sup>b</sup>	42.19 <sup>b</sup>	42.06 <sup>b</sup>	43.20 <sup>bc</sup>	44.89 <sup>b</sup>	1.34
	a*	26.67 <sup>a</sup>	25.33 <sup>b</sup>	25.15 <sup>bc</sup>	24.71 <sup>b</sup>	24.12 <sup>bc</sup>	22.86 <sup>bc</sup>	24.46 <sup>bc</sup>	23.01 <sup>b</sup>	0.69
	b*	21.89	20.73	19.75 <sup>b</sup>	20.63	19.14	18.71	19.54	19.75	1.36
Day 42	L*	49.24 <sup>ab</sup>	47.90 <sup>ab</sup>	47.16 <sup>ab</sup>	48.83 <sup>ab</sup>	46.34 <sup>ab</sup>	46.18 <sup>ab</sup>	47.09 <sup>bc</sup>	49.79 <sup>a</sup>	1.34
	a*	20.73 <sup>b</sup>	20.66 <sup>c</sup>	21.50 <sup>d</sup>	21.63 <sup>c</sup>	21.22 <sup>c</sup>	21.39 <sup>bc</sup>	22.15 <sup>c</sup>	21.67 <sup>b</sup>	0.69
	b*	17.92	17.84	18.10 <sup>b</sup>	19.14	17.66	18.46	18.43	19.62	1.36
Day 56	L*	43.62 <sup>b</sup>	43.78 <sup>b</sup>	42.83 <sup>b</sup>	46.21 <sup>b</sup>	41.99 <sup>b</sup>	42.60 <sup>b</sup>	42.38 <sup>c</sup>	46.47 <sup>b</sup>	1.34
	a*	21.76 <sup>b</sup>	20.85 <sup>c</sup>	22.22 <sup>d</sup>	22.84 <sup>c</sup>	21.03 <sup>c</sup>	20.71 <sup>c</sup>	22.52 <sup>c</sup>	21.45 <sup>b</sup>	0.69
	b*	18.35	17.84	17.97 <sup>b</sup>	20.08	17.49 <sup>y</sup>	17.55	18.10	19.23	1.36

<sup>a-d</sup>Means with the same letter within a column are not significantly different (P < 0.05)

<sup>w-z</sup>Means with the same letter within a row are not significantly different (P < 0.05)

<sup>e</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels

<sup>f</sup>SEM = Standard error of the mean.

<sup>g</sup>Frozen (-20°C) patties stored in plastic-lined cardboard boxes

other treatments at d 0. Lipid blend treatment and storage day had no effect on yellowness values past d 0.

### **pH determination**

The pH of refrigerated patties was not affected ( $P > 0.05$ ) by lipid blend treatment on day 0. The only treatment affected by storage day was OO 10, which was lower ( $P < 0.05$ ) on d 9 (5.27) than on d 0 (5.43; data not shown). The pH of frozen patties was highest ( $P < 0.05$ ) on d 28 for all treatments (5.81-5.89). Treated beef patty pH did not differ ( $P > 0.05$ ) between d 0 and d 56 (5.43-5.54; data not shown).

### **Cooked internal color, cook yield, Allo-Kramer shear, and dimensional changes**

Means for cook yield percentages, Allo-Kramer shear values, cooked internal color values and dimensional changes are presented in Table 8. Internal  $L^*$  (lightness) values of cooked patties were slightly higher ( $P < 0.05$ ) for SO 20, OO 10, and CO 20 than for either of the control patties. No treatment patties were higher than the controls for  $a^*$  (redness) values. Values for  $b^*$  (yellowness) were lowest for the 10 and 20% control patties and highest for the CO 10 and 20% treated patties.

Percentage cook yield was higher ( $P < 0.05$ ) for patties containing 10% fat than those containing 20% fat (CT, SO, OO). CO 10 had higher cook yields compared to CO 20, but they were not significantly different ( $P > 0.05$ ). Similar results were reported by Troutt and others (1992) which determined that beef patties with lower fat percentages tended to have higher cook yields. The controls had the highest cook yields within each fat level (10 and 20%), with CT 10 being significantly higher ( $P < 0.05$ ) than CO 10 and

Table 8. Least squares means for cook yield, shear values, cooked CIE L\*, a\* and b\* values, and dimensional changes of beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%)

	CT		SO		OO		CO		SEM <sup>h</sup>
	Fat level (%) <sup>f</sup>								
	10	20	10	20	10	20	10	20	
% Cook Yield	74.84 <sup>a</sup>	72.43 <sup>bc</sup>	74.14 <sup>ab</sup>	69.97 <sup>d</sup>	73.83 <sup>abc</sup>	69.65 <sup>d</sup>	72.22 <sup>bc</sup>	71.75 <sup>cd</sup>	0.62
Shear Value <sup>g</sup>	30.69 <sup>a</sup>	26.19 <sup>bc</sup>	27.59 <sup>b</sup>	23.02 <sup>de</sup>	30.46 <sup>a</sup>	24.27 <sup>cd</sup>	28.17 <sup>b</sup>	21.64 <sup>e</sup>	0.60
L*	51.24 <sup>b</sup>	51.55 <sup>b</sup>	52.78 <sup>ab</sup>	54.53 <sup>a</sup>	54.16 <sup>a</sup>	53.10 <sup>ab</sup>	53.08 <sup>ab</sup>	54.37 <sup>a</sup>	0.49
a*	4.11 <sup>ab</sup>	4.09 <sup>ab</sup>	3.87 <sup>cb</sup>	3.80 <sup>cb</sup>	3.62 <sup>c</sup>	3.85 <sup>cb</sup>	4.24 <sup>a</sup>	4.06 <sup>ab</sup>	0.08
b*	15.82 <sup>d</sup>	16.24 <sup>cd</sup>	16.57 <sup>cd</sup>	17.10 <sup>ab</sup>	16.40 <sup>cd</sup>	17.08 <sup>ab</sup>	17.45 <sup>a</sup>	17.48 <sup>a</sup>	0.15
Patty diameter change, mm	-10.99 <sup>b</sup>	-12.73 <sup>b</sup>	-12.33 <sup>b</sup>	-14.77 <sup>a</sup>	-12.91 <sup>ab</sup>	-12.59 <sup>b</sup>	-11.81 <sup>b</sup>	-12.70 <sup>b</sup>	0.45
Patty thickness change, mm	-0.08 <sup>abc</sup>	-0.28 <sup>ab</sup>	-0.35 <sup>ab</sup>	-0.65 <sup>a</sup>	-0.13 <sup>abc</sup>	-0.43 <sup>a</sup>	+0.38 <sup>c</sup>	+0.26 <sup>bc</sup>	0.14

<sup>a-e</sup> Means in a row with different superscripts are significantly different (P < 0.05)

<sup>f</sup> Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels

<sup>g</sup> Shear values reported as Newtons/gram

<sup>h</sup> SEM = Standard error of the mean

CT 20 being significantly higher ( $P < 0.05$ ) than SO 20 and OO 20. Similar findings were reported by Dzudie and others (2004) and Babji and others (1998), who observed that beef patties formulated with 20% maize and ground-nut oils lost more weight due to cooking than control patties.

Overall cooked patty means for Allo-Kramer shear, an objective measurement of instrumental tenderness, ranged from 21.64 N/g to 30.69 N/g. All 20% fat patties were lower for Allo-Kramer shear values (more tender) ( $P < 0.05$ ) than the corresponding 10% fat patties for each lipid blend treatment, suggesting that patties with a higher fat content were more tender regardless of fatty acid profile. These findings are supported by work (Park and others 1989) in beef/pork frankfurters supplemented with high oleic sunflower oil. The authors saw much larger differences in tenderness as determined by texture profile analysis and sensory evaluation between high fat and low fat frankfurters than between control and supplemented frankfurters. The authors concluded that amount of fat had more influence in texture than individual fatty acid composition. For all 10% treatments SO 10 and CO 10 were more tender ( $P < 0.05$ ) than OO 10 and CT 10. For 20% treatments SO 20 and CO 20 were both more tender ( $P < 0.05$ ) than OO 20 and the 20 control. Overall, all lipid blend treatment patties were as tender ( $P > 0.05$ ) or more tender ( $P < 0.05$ ) than the control patties. Dzudie and others (2004) also observed that beef patties formulated with vegetable oils were softer as determined by textural analysis.

SO 20 experienced a greater loss ( $P < 0.05$ ) in diameter (14.77 mm) from cooking than all other treatments, excluding OO 10. All other treatments were similar in their diameter change. CO 10 and 20 were the only treatments to increase in patty thickness after cooking.

### **Sensory evaluation**

Descriptive sensory data (Table 9) indicated that the lipid blend treatment used in the treatment patties had an effect ( $P < 0.05$ ) on sensory texture, but no effect ( $P > 0.05$ ) on aromatics, basic tastes, aftertastes, mouthfeels, and after mouthfeels. Control and treatment

patties at both fat levels affected juiciness and sandy/gritty texture ( $P < 0.05$ ). Patties manufactured with SO 20 received the highest rating for juiciness (7.67) and were significantly higher ( $P < 0.05$ ) than CT 10 (5.60) and OO 10 (5.87) patties. Differing results to the ones in this study were observed by Shiota and others (1995), who found that sensory scores for texture and aroma tended to decline as the amount of unsaturated oil (palm super-olein, soybean) increased in beef patties.

Patties formulated at 10% fat tended to be less juicy ( $P < 0.05$ ) than those containing 20% fat. Similar findings for comparisons of fat levels were reported by Troutt and others (1992) and Berry and Leddy (1984). For Sandy/Gritty texture the only treatment higher than the control ( $P < 0.05$ ) was CO 10. Aromatics associated with rancidity, such as cardboard, painty, and fishy were only slightly detected and were not affected by treatment. No attributes associated with rancidity were above 0.67 on a 15 point Spectrum scale.

Table 9. Least squares means of descriptive attribute sensory panel scores (combined) for textures, aromatics, basic tastes, mouthfeels, aftertastes, and after mouthfeels of cooked beef patties formulated with a mixture of beef tallow (57%) and mono- or polyunsaturated oils (43%) at 10% and 20% fat levels

Attributes	CT		SO		OO		CO		SEM
	Fat level (%) <sup>d</sup>								
	10	20	10	20	10	20	10	20	
<b>Textures</b>									
Juiciness	5.60 <sup>c</sup>	6.20 <sup>abc</sup>	6.06 <sup>abc</sup>	7.67 <sup>a</sup>	5.87 <sup>bc</sup>	6.57 <sup>abc</sup>	6.47 <sup>abc</sup>	7.33 <sup>ab</sup>	0.40
Sandy/gritty	3.33 <sup>ab</sup>	3.00 <sup>b</sup>	3.50 <sup>ab</sup>	3.91 <sup>ab</sup>	3.42 <sup>ab</sup>	3.42 <sup>ab</sup>	3.25 <sup>ab</sup>	4.08 <sup>a</sup>	0.23
<b>Aromatics</b>									
Cooked Beef Fat	6.00	6.53	5.80	5.40	6.00	5.53	5.87	5.87	0.29
Cooked Beef Lean	1.42 <sup>ab</sup>	2.00 <sup>a</sup>	1.50 <sup>ab</sup>	1.17 <sup>b</sup>	1.41 <sup>ab</sup>	1.08 <sup>b</sup>	1.33 <sup>ab</sup>	1.41 <sup>ab</sup>	0.18
Oil	2.25	2.75	2.75	3.58	2.91	3.42	2.75	3.58	0.31
Cardboard	0.67	0.20	0.07	0.33	0.13	0.53	0.07	0.20	0.17
Painty	0.00	0.00	0.00	0.00	0.67	0.00	0.67	0.00	0.07
Fishy	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.03
Browned	0.40	0.33	0.33	0.20	0.40	0.13	0.47	0.20	0.15
<b>Tastes</b>									
Salt	0.80	0.93	1.07	0.80	0.87	0.87	0.93	0.80	0.11
Sour	2.47	2.53	2.60	2.67	2.47	2.80	2.67	2.87	0.16
Bitter	2.33	2.33	2.13	2.40	2.27	2.33	2.40	2.67	0.16
<b>Aftertastes</b>									
Sour	2.33	2.20	2.27	2.33	2.13	2.47	2.27	2.53	0.13
Bitter	2.20	2.20	2.20	2.13	2.07	2.13	2.07	2.40	0.10
Browned	0.20	0.13	0.20	0.20	0.27	0.00	0.27	0.20	0.11
<b>Mouthfeels</b>									
Metallic	2.60	2.47	2.47	2.73	2.53	2.53	2.67	2.53	0.14
Astringent	2.87	2.87	2.73	2.80	3.07	3.20	2.87	3.00	0.14
Oily/Greasy	1.47	2.00	1.53	2.33	1.60	2.33	1.80	2.20	0.26
Fatty Coating	0.80	1.20	1.27	0.93	1.07	0.80	0.93	0.93	0.19
<b>After Mouthfeels</b>									
Metallic	2.47	2.20	2.27	2.13	2.20	2.40	2.27	2.47	0.13
Astringent	2.47	2.27	2.27	2.47	2.73	2.40	2.40	2.67	0.19
Oily/Greasy	1.00	1.27	1.13	1.60	1.40	1.53	1.13	1.60	0.21
Fatty Coating	0.73	1.00	0.93	0.53	0.73	0.53	0.80	0.67	0.15

<sup>a-c</sup> Means in a row with different superscripts are significantly different ( $P < 0.05$ )

<sup>d</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels

### Fatty acid composition

Least square means for fatty acid composition of manufactured lipid blends are presented in Table 10. Lipid blends with beef tallow and olive oil were highest ( $P < 0.05$ ) in palmitic (16:0), palmitoleic (16:1), and stearic acid (18:0), and were lowest for linoleic (18:2), contained no linolenic acid (18:3), and had the lowest ( $P < 0.05$ ) unsaturated to saturated fatty acid ratio (1.98). High oleic safflower and beef tallow blends were highest ( $P < 0.05$ ) in oleic acid (18:1; 57.01%) and unsaturated to saturated fatty acid ratio (2.39). Corn oil and beef tallow blends were highest ( $P < 0.05$ ) for linoleic acid (18:2) and polyunsaturated to saturated fatty acid ratio (0.83), but were lowest ( $P < 0.05$ ) for oleic acid (18:1). Myristic acid (14:0) was present at a range of 1.23 to 1.27, but was not affected across lipid blend treatments.

In cooked beef patties (Table 11), significant differences in lipid blend treatment patties were observed for six fatty acids (C14:0, C16:0, C18:0, C18:1, C18:2, and C18:3). Control beef patties at both fat levels (10 and 20%) were higher ( $P < 0.05$ ) than all other treatments for myristic (14:0) and palmitic acid (16:0), which are both responsible for increasing total cholesterol and low density lipoprotein cholesterol in humans (Kris-Etherton and Yu 1997). The controls were also higher ( $P < 0.05$ ) for stearic acid (18:0), which, although saturated, has a neutral cholesterolemic effect (Yu and others 1995), compared to all treatments except for OO 10. Oleic acid (18:1) was the major fatty acid found in all lipid blend patty treatments, accounting for at least 36%, with SO 20 containing 50.27%, the highest ( $P < 0.05$ ) of all treatments. As expected, CO 10 and CO 20 contained significantly more ( $P < 0.05$ ) linoleic acid (18:2) (14.40% and 19.46%, respectively) than all other treatments while the controls had the lowest (3.24% and 2.67%, respectively). Conjugated linoleic acid (CLA) isomer *cis*-9, *trans*-11, a beneficial fatty acid produced as a byproduct of rumen biohydrogenation that is known to have anticarcinogenic (De la Torre and others 2006) and antiatherogenic (Lee and others 1994) effects, was present in all beef patties, though its concentration was not diminished ( $P > 0.05$ ) by use of a lipid blend treatment. Addition of a beef tallow/corn oil blend to achieve 10 and 20%



fat was successful in raising the PUFA:SFA ratio from 0.1 and 0.09 in the 10 and 20% fat controls, respectively, to 0.41 and 0.62, respectively, within the recommended range of 0.4 - 1.0 (Wood and others 2004a). MUFA:SFA ratios in SO 10 and 20 (1.29 and 1.58, respectively) and OO 10 and 20 (1.17 and 1.42, respectively) were significantly higher ( $P < 0.05$ ) compared to the controls (0.88 and 0.92, respectively). Increased MUFA:SFA ratios have been shown to decrease LDL and total cholesterol in humans (Mattson and Grundy 1985).

### **Conclusions**

The inclusion of beef tallow/edible oil lipid blends into beef patties was achieved without compromising physical or textural characteristics. Sensory analysis showed little difference between control beef patties and those manufactured with a lipid blend treatment. Lipid blend treatment patties were shown to have the same oxidative stability as control patties when manufactured with a rosemary extract antioxidant. High oleic safflower oil and olive oil lipid blend treatments were successful in raising the MUFA/SFA ratio, while corn oil lipid blend treatments displayed more optimal PUFA/SFA ratios. Overall results of this study suggest that replacement of beef fat by a beef tallow/edible oil blend is feasible for production of a beef product with a healthier image.

Table 10. Least squares means for fatty acid composition of lipid blends formulated with beef tallow (57%) and mono- or polyunsaturated oils (43%)

Fatty Acid		Beef Tallow/High Oleic Safflower Oil	Beef Tallow/Olive Oil	Beef Tallow/Corn Oil	SEM <sup>d</sup>
Myristic	14:0	1.27	1.21	1.23	0.02
Palmitic	16:0	14.81 <sup>c</sup>	18.23 <sup>a</sup>	17.38 <sup>b</sup>	0.10
Palmitoleic	16:1	1.35 <sup>b</sup>	1.76 <sup>a</sup>	1.36 <sup>b</sup>	0.01
Stearic	18:0	10.27 <sup>b</sup>	11.36 <sup>a</sup>	10.35 <sup>b</sup>	0.10
Oleic	18:1	57.01 <sup>a</sup>	55.48 <sup>b</sup>	37.15 <sup>c</sup>	0.24
Linoleic	18:2	8.05 <sup>b</sup>	5.65 <sup>c</sup>	25.45 <sup>a</sup>	0.13
Linolenic	18:3	0.16 <sup>a</sup>	0.00 <sup>c</sup>	0.12 <sup>ab</sup>	0.04
UFA:SFA Ratio		2.39 <sup>a</sup>	1.98 <sup>c</sup>	2.13 <sup>b</sup>	0.03
PUFA:SFA Ratio		0.30 <sup>b</sup>	0.17 <sup>c</sup>	0.83 <sup>a</sup>	0.01

<sup>a-c</sup>Means in a row with different superscripts are significantly different ( $P < 0.05$ )

<sup>d</sup>SEM = Standard error of the mean

Table 11. Least squares means for fatty acid composition (% of total fatty acids) of cooked beef patties formulated with a blend of beef tallow (57%) and mono- or polyunsaturated oils (43%)

Fatty Acid		CT		SO		OO		CO		SEM <sup>k</sup>
		10	20	10	20	10	20	10	20	
Myristic	14:0	3.13 <sup>a</sup>	3.23 <sup>a</sup>	2.26 <sup>b</sup>	1.81 <sup>c</sup>	2.35 <sup>b</sup>	1.86 <sup>c</sup>	2.21 <sup>b</sup>	1.79 <sup>c</sup>	0.05
Palmitic	16:0	23.13 <sup>a</sup>	22.86 <sup>a</sup>	19.61 <sup>c</sup>	17.45 <sup>d</sup>	21.55 <sup>b</sup>	19.86 <sup>c</sup>	20.90 <sup>b</sup>	19.21 <sup>c</sup>	0.17
Palmitoleic	16:1	2.14	3.35	2.16	1.81	2.43	2.13	2.15	1.79	0.36
Stearic	18:0	13.57 <sup>a</sup>	13.52 <sup>a</sup>	12.01 <sup>bc</sup>	10.93 <sup>e</sup>	12.38 <sup>a</sup>	11.52 <sup>cd</sup>	12.06 <sup>bc</sup>	10.94 <sup>de</sup>	0.12
Oleic	18:1	37.29 <sup>d</sup>	37.56 <sup>d</sup>	46.12 <sup>b</sup>	50.27 <sup>a</sup>	43.76 <sup>c</sup>	48.09 <sup>b</sup>	36.74 <sup>d</sup>	36.09 <sup>e</sup>	0.41
Linoleic	18:2	3.24 <sup>f</sup>	2.67 <sup>f</sup>	6.02 <sup>cd</sup>	6.80 <sup>c</sup>	4.73 <sup>e</sup>	5.30 <sup>de</sup>	14.40 <sup>b</sup>	19.46 <sup>a</sup>	0.24
Linolenic	18:3	0.41 <sup>ab</sup>	0.56 <sup>a</sup>	0.27 <sup>b</sup>	0.29 <sup>b</sup>	0.25 <sup>b</sup>	0.29 <sup>b</sup>	0.23 <sup>b</sup>	0.34 <sup>b</sup>	0.05
CLA c-9, t-11		0.21	0.12	0.19	0.21	0.19	0.13	0.14	0.15	0.05
PUFA:SFA Ratio <sup>h</sup>		0.10 <sup>ef</sup>	0.09 <sup>f</sup>	0.19 <sup>cd</sup>	0.24 <sup>c</sup>	0.14 <sup>de</sup>	0.18 <sup>d</sup>	0.41 <sup>b</sup>	0.62 <sup>a</sup>	0.05
MUFA:SFA Ratio <sup>i</sup>		0.88 <sup>g</sup>	0.92 <sup>g</sup>	1.29 <sup>c</sup>	1.58 <sup>a</sup>	1.17 <sup>d</sup>	1.42 <sup>b</sup>	1.02 <sup>ef</sup>	1.11 <sup>de</sup>	0.02

<sup>a-e</sup>Means in a row with different superscripts are significantly different (P < 0.05)

<sup>h</sup>PUFA:SFA = Polyunsaturated fatty acid to saturated fatty acid ratio

<sup>i</sup>MUFA:SFA = Monounsaturated fatty acid to saturated fatty acid ratio

<sup>j</sup>Fat levels represent a mixture of beef fat present in the raw materials (6%) and manufactured lipid blends added at 4% and 14%, respectively to achieve the desired 10% and 20% fat levels

<sup>k</sup>SEM = Standard error of the mean

## CHAPTER IV

### CONCLUSIONS

The use of lipid blends containing beef tallow, edible oil, and a rosemary extract antioxidant in this study was successful in altering the fatty acid composition of beef patties. MUFA/SFA ratios were increased through the use of high oleic safflower and olive oil lipid blend treatments, while corn oil lipid blend treatments were shown to have increased PUFA/SFA ratios. Descriptive sensory analysis showed little differences between treatment patties and control patties. In addition, the oxidative stability of patties containing greater amounts of unsaturated fats present in the lipid blends was shown to be no different than that of the control patties. Based on the results of this study it is reasonable to suggest that a beef tallow/edible oil lipid blend can be used to replace beef fat to alter the fatty acid profile of beef patties.

Furthermore, the lipid blend concept used in this study could be used in further research regarding the impact of beef, as well as other muscle foods, on human health. Although, the vegetable oils used in this study were high in MUFA (high oleic safflower oil, olive oil) and overall PUFA (corn oil) other oils that are high in n-3 fatty acids (canola oil, linseed oil) could be used as well. Marine oils, which contain highly unsaturated, long-chain n-3 fatty acids known to be beneficial to human health, could also be used. As research into the effects of different fatty acids on human health and diseases progresses, the lipid blend concept has the ability to allow the use of many different oils to achieve a nutritionally beneficial fatty acid composition. Incorporation of an emulsifier into the system to achieve more efficient binding of the highly unsaturated oils is also a possibility.

While beef patties were the only product used in this study, the system employed to incorporate the lipid blends into the product (bowl chopper) could also be used to manufacture different processed muscle foods. Cured, dried, fermented and fresh coarse chopped sausage products are a possibility, as are fully emulsified frankfurters. These chopped and emulsified

products present challenges as far as incorporation of high amounts of low melting point unsaturated fats because of the longer chopping times, and the resulting rise in product temperature, necessary to produce them. As mentioned earlier, an emulsifier could be used to increase the fat binding ability of the food system. These products, often high in fat and seen as unhealthy by consumers could benefit greatly from a healthier image.

The use of different production systems could be possible to allow incorporation of lipid blends into a wider range of products. Mixing lipid blends with chopped and formed and formed emulsified products is a possibility. The use of an injector to deliver lipid blends into whole muscle products such as roasts would not only increase nutritional value, but quality as well.

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**APPENDIX A**

Figure 1. Ballot used for descriptive sensory analysis of beef patties

Sample	Textures		Aromatics									Tastes			Mouthfeels				Aftertastes			After Mouthfeels				
	Juiciness	Sandy/Gritty	Cooked Beef Lean	Cooked Beef Fat	Oil	Olive Oil	Cardboard	Painty	Fishy	Liver	Browned	Salt	Sour	Bitter	Metallic	Astringent	Oily/Greasy	Fatty Coating	Sour	Bitter	Browned	Metallic	Astringent	Oily/Greasy	Fatty Coating	
W/ U																										
1																										
2																										
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## APPENDIX B

<b>Attribute</b>	<b>Description</b>
<b>Textures</b>	
Juiciness	The amount of juice/moisture perceived in the mouth.
Mealy/Grainy	The amount of rough, grain-like residues perceived.
<b>Aromatics</b>	
Cooked beef lean	The aromatic associated with cooked beef muscle meat.
Cooked beef fat	The aromatic associated with cooked beef fat.
Oil	The aromatic associated with cooked vegetable oils.
Olive oil	The aromatic associated with cooked olive oil.
Cardboard	The aromatic associated with slightly stale beef, refrigerated for a few days only and associated with wet cardboard and stale oils and fats.
Painty	The aromatic associated with rancid oil and fat.
Fishy	The aromatic associated with some rancid fats and oils.
Liver	The aromatic associated with beef liver and/or kidney.
Browned	The aromatic associated with the outside of grilled or broiled beef.
<b>Tastes</b>	
Salt	The taste stimulated by sodium salts, such as sodium chloride and sodium glutamate, and in part by other salts, such as potassium chloride.
Sour	The taste stimulated by acids, such as citric, malic, phosphoric, etc.
Bitter	The taste stimulated by substances such as quinine, caffeine and hop bitters.
<b>Mouthfeels</b>	
Metallic	The sensations on the tongue associated with metals such as iron or copper.
Astringent	The shrinking or puckering of the tongue surface caused by substances such as tannin or alum.
Oily/greasy	Amount of oil left on fat surfaces.
Fatty coating	Amount of fat left on mouth surfaces.

## APPENDIX C

### LIPID EXTRACTION AND QUANTIFICATION OF FATTY ACID BY GAS CHROMATOGRAPHY

#### EQUIPMENT:

Balance / Scale	Graduated cylinder
50 ml Polypropylene centrifuge tubes w/ screw caps	Kimwipes®
Refrigerator / cooler	Vacuum
Homogenizer	Vortex mixer
500 ml side-arm flask with hose	Centrifuge
Heated water bath	Nitrogen evaporator
Gas chromatograph	Pasteur pipete
CP Sil-88 capillary column (100 meter length preferred)	Timer
Glass filter assembly vial	Glass scintillation vial
2.4 cm Glass filters (0.2 $\mu$ )	2.0 ml autosampler vial
30 ml glass tubes	
15 ml glass tubes	

#### REAGENTS:

Nitrogen gas  
 HPLC grade Hexane  
 FAME standards (Nu-Chek-Prep, Inc. Elysian, MN)  
 Chloroform:Methanol (2:1, v/v)  
 14% boron trifluoride in methanol  
 Saturated NaCl  
 Na<sub>2</sub>SO<sub>4</sub>

#### Solutions:

0.5 N KOH in MeOH: 2.81 g KOH in 100 ml MeOH  
 It takes approximately 10 minutes to dissolve.

Saturated NaCl: 31.7 g NaCl in 100 ml dd H<sub>2</sub>O

0.74% KCl: 7.4 g KCl in 1 liter ddH<sub>2</sub>O

Nu-Chek Prep, Inc. 1-800-521-7728  
 GLC #68D and #211 (FAME Standards)

#### PROCEDURE:

##### Extraction of Total Lipids

1. Weigh ~100 mg adipose tissue (i.e. chicken or beef fat), or 0.5-1 g muscle or 2-5 ml plasma or 2 g digesta and add to a labeled 50 ml tube (plastic/glass, with cap). Record the exact sample weight. (500 mg of fat can be used, but only saponify 100  $\mu$ l.)

NOTE: Some plastics are attacked by chloroform/methanol.

Polypropylene is generally safe. Polyethylene is not. **50 ml polypropylene centrifuge tubes for cell culture are recommended.**

2. Preparation of external control: Measure 5 mg each of 14-0, 16-0 and 18-0 triglyceride standards. Record the exact weights of each.
3. Add 5.0 ml of chloroform:methanol ( $\text{CHCl}_3:\text{CH}_3\text{OH}$ , 2:1, v/v) to each tube, including the external controls.
4. Homogenize each sample with Polytron (or similar) homogenizer on medium setting for ~30 seconds. After homogenization, rinse the probe with  $\text{CHCl}_3:\text{CH}_3\text{OH}$  until final volume in the tube is ~15 ml. Rinse the probe with warm water. Spray the probe with clean water into waste beaker. Rinse with  $\text{CHCl}_3:\text{CH}_3\text{OH}$  into another waste beaker. Dry with Kimwipes.  
NOTE: If using pure lipid (fat), skip homogenization step and bring final volume in each tube to ~15 ml with chloroform:methanol.
5. Let sample sit for at least 30 minutes to extract the lipids. If stopping at this point, flush the tube with nitrogen, cap and store at 4°C.
6. Filter homogenate into a new 50 ml centrifuge tube using a glass microanalysis filter holder assembly with disposable 2.4 cm glass fiber filters. Place glass fiber filter onto assembly rough side up. Rinse homogenate tube 2 times with  $\text{CHCl}_3:\text{CH}_3\text{OH}$  using a squeeze bottle. Also rinse filter funnel 1-2 times with  $\text{CHCl}_3:\text{CH}_3\text{OH}$ . (**See detailed filtering instructions below.**)
7. Add  $\text{CHCl}_3:\text{CH}_3\text{OH}$  to the filtered homogenate until you have a final volume of 20 ml. Measure with a graduated cylinder and replace in tube.
8. Add 8 ml of 0.74% KCl and vortex 1 minute.
9. Let sit 2 hours to separate phases or centrifuge at ~600 x g until you get two distinct phases. If stopping at this point, flush with nitrogen, cap and store at 4°C overnight.
10. Carefully remove ALL of the upper phase (non-lipid portion) using a pasteur pipet and discard. If you want to stop at this point, flush with nitrogen, cap and store at -20°C.
11. Transfer all lower phase (contains lipid) to 30 ml glass tubes. Rinse 50 ml tube 2-3 times with  $\text{CHCl}_3:\text{CH}_3\text{OH}$  into the 30 ml glass tube.
12. Evaporate the sample to dryness with nitrogen using the N-EVAP analytical evaporator.
13. If fat was extracted from a 500 mg sample in Step 1, collect 100  $\mu$ l after evaporation and use for saponification process.

**Filtering Lipid Samples:**

1. Set up filter holder assembly consisting of 15 ml filter tube, fritted glass filter with stopper, aluminum clamp, 500 ml side-arm flask and vacuum hose.
2. Place 50 ml tube (marked at 20 ml) in the 500 ml side arm flask.
3. Replace glass filter and stopper. Make sure stem of filter is inside 50 ml tube.
4. Place 2.4 cm glass fiber filter on top of glass filter (rough side facing up).
5. Replace 15 ml filter tube and clamp it.
6. Turn on vacuum. Make sure vacuum hose is connected to flask and lab vacuum.
7. Pour sample into 15 ml filter tube. Avoid filtering too quickly. Add the rinse liquid before the filter tube is dry to prevent the filter from clogging.
8. Rinse 50 ml tube with Chloroform:Methanol 2:1 (a few squirts) and pour into 15 ml tube. Rinse side of 15 ml filter tube with Chloroform:Methanol 2:1 (a few squirts).
9. Add Chloroform:Methanol 2:1 to bring the volume in the second 50 ml tube to 20 ml.
10. Remove 2<sup>nd</sup> 50 ml tube, cap and set to the side.
11. Remove and discard glass fiber filter.
12. Make sure 15 ml tube is clean. Run Kimwipe through it to clean it.

**Saponification and Methylation of Lipids:**

1. Add 1 ml of 0.5 N KOH in methanol. Heat in 70°C bath for 10 minutes.
2. Add 1 ml of 14% BF<sub>3</sub> (boron trifluoride) in methanol, flush with N<sub>2</sub>, cap loosely, and place in 70°C water bath for 30 minutes.
3. Remove the tubes and allow them to cool for 5 minutes. Add 2 ml HPLC grade hexane and 2 ml saturated NaCl. Vortex for 1 minute.
4. Pipet off upper hexane layer with transfer pipet and place in 20 ml tube containing ~800 mg Na<sub>2</sub>SO<sub>4</sub>. Add 2 more ml of hexane to the tube with saturated NaCl. Vortex, allow to settle and pipet the upper hexane layer into the 20 ml tube with Na<sub>2</sub>SO<sub>4</sub>. You should now have approx. 4 ml of hexane in this tube. Vortex this tube briefly. The Na<sub>2</sub>SO<sub>4</sub> removes any moisture from the hexane. Allow tube to stand until the Na<sub>2</sub>SO<sub>4</sub> settles and the hexane is clear.
5. Pipet the hexane into the labeled glass scintillation vial. Be careful not to pull any of the Na<sub>2</sub>SO<sub>4</sub> crystals into the pipet. It is better to leave some of the hexane behind than to get the crystals because they can clog up the capillary column.

6. Add 1 ml hexane to the 20 ml tube with Na<sub>2</sub>SO<sub>4</sub> in it. Vortex briefly and let tube stand as described above. Transfer the hexane to the glass scintillation vial.
7. Evaporate the hexane completely with the N-EVAP.
8. Reconstitute the lipid with HPLC grade hexane as follows: 500 µl hexane for plasma, 500 µl hexane for digesta, 500 µl hexane for meat, 500 µl hexane for liver or any other tissue besides fat, 1 ml hexane for subcutaneous or intramuscular fat. Swirl vial gently to mix.
9. Transfer to a labeled 2.0 ml autosampler vial. Pipet (100 µl for digesta, plasma, muscle, liver or tissues other than fat; or 400 µl for fat) this solution into a 2-ml autosampler vial containing 1.6 ml of HPLC-grade hexane containing 5 mg C12 FAME internal standard (FAME 12:0 @ 12.5 mg/ml). An autosampler glass insert should be used to hold samples that are 100 µl. As with the external standard, the 12:0 internal standard is appropriate for meat samples since they generally contain very little 12:0 lipids and thus present an independent peak on the GC. Alternate standards may be desirable for other sources of lipids.
10. Inject 0.5 to 1.0 µl into the gas chromatograph.

#### Calculations:

1. List the areas for your external standards and your internal standard (IS). Calculate the F<sub>x</sub> for each of the fatty acids in the external standard.

$$F_x = (\text{Area IS} / \text{Amount IS}) \div (\text{Area FA}_{\text{ex}} / \text{Amount FA}_{\text{ex}})$$

Where: F<sub>x</sub> = Correction for loss during extraction and methylation  
 Area IS = Area of the internal standard (12:0)  
 Amount IS = Amount of internal standard added (5 mg)  
 Area FA<sub>ex</sub> = Area for each fatty acid in the external standard  
 Amount FA<sub>ex</sub> = Amount of each external fatty acid added (5 mg)

You will generate three F<sub>x</sub> values (14:0, 16:0 and 18:0). You can use the F<sub>x</sub> that is closest to the FAME being evaluated or just use the simple average of all three F<sub>x</sub> values.

2. Enter data from the Star Program printouts in the table on the next page. Use the FAME Standards printout to identify the major fatty acid peaks. You may have some very small peaks that cannot be identified. Add the areas of these peaks together and enter the sum in the unidentified row.
3. Use the following equation to calculate the actual quantity of each fatty acid. Enter the calculated values in the Fatty Acid table.

$$\text{Amount FA (g/100g)} = F_x \times \frac{(\text{Area FA}) \times (\text{Amount IS})}{(\text{Area IS}) \times (\text{Amount Sample})} \times 100$$



4. Calculate the area percentage of each fatty acid and enter in the Fatty Acid table.

$$\text{Area Percentage} = \frac{\text{g fatty acid}}{\text{Total g fatty acids}} \times 100$$

## GC PROTOCOL

### TO TURN ON THE FLAME:

Go to **FILE**

#### ACTIVATE METHOD

Find **FAME NEW TEMP** and click on it.

#### Shortcut:

There should be a button on the top that says **GAS SAVER** next to that there should be a small button that looks like an open file to the right. Click on this. This will open the methods list. Find **FAME NEW TEMP** and click on it. Use the glass Petri dish to check to see if the flame is on.

### SAMPLE LIST:

Click on **FILE** → **NEW SAMPLE LIST**

Use the date with dashes for the name → click and save at the bottom of the screen.

Load the vials in the carrousel.

Click on the **CARROUSEL BUTTON** on the bottom of the screen.

A new window will open. Click on **APPEND**. The autosampler will begin to scan the carrousel and enter all the vials for you. All you need to do is rename all the samples. Use the arrow keys to go up or down. Do not change anything in the other columns. When you are done renaming the samples and the flame is lit begin the sequence by clicking **BEGIN** on the bottom left. It will open a new window with the Varian machine number and operator name. Enter your name as the operator and click **OK**. Then another window will open asking you if this is the correct method. Click **OK**.

When the samples are finished running, put the GC back in **GAS SAVER METHOD**. Go to the methods activation screen and find the **GAS SAVER METHOD**. Click on it. Then turn off the **Hydrogen** (Red) and the **Air** (yellow). **DO NOT TOUCH THE HELIUM (BLUE)!**

### TO PRINT FILES:

Before you start opening files, please minimize the system setup screen. This will prevent you from accidentally shutting down a run.

There is a sequence of buttons on the left of the screen that run up and down. The 4<sup>th</sup> button is **STANDARD REPORTS**. Click on this button. It will open a new window. The name of the folder should be **DATA**. If it is not, you need to go up to find the data folder. Find your samples click on one and then click **OPEN FILE** (the bottom of the screen).

**Change the Chromatogram for easier reading:**

Click on **OPTIONS** at the top of the screen. Change the start time to **5.00** and the end time to **43.00**. Then **UNCHECK** auto scale. Click **OK**.

Click on the **PRINT BUTTON** at the top of the screen. Make sure to check both the Chromatogram and the results. Click **OK**. When you open the next file it will ask if you want to save the changes. Click **YES**.

When you get finished using the computer leave the system setup screen minimized.

**TO ADD OR REMOVE PEAKS:**

There is a sequence of buttons on the left of the screen that run up and down. The 3<sup>rd</sup> button is **VIEW/EDIT CHROMATOGRAM**. Click on this button. It will open a new window. The name of the folder should be **DATA**. If it is not, you need to go up to find the data folder. Find your samples click on one and then click **OPEN FILE** (the bottom of the screen). You will get a blue Chromatogram.

Click on **EDIT METHOD** on the top of the screen. Go to **INTEGRATION PARAMETERS**. You want to change the **INITIAL PEAK REJECTION VALUE**, right side in the middle. This is the lowest value of peaks it will find. Click **SAVE** when done. Now you need to go the top of the screen and click **RESULTS** and then **REINTEGRATE NOW**. The chromatogram should flash and peaks should appear or disappear depending on how you set the range. You will need to **CLEAR** between each sample.

You do not need to save them. They are automatically saved with the changes. The computer will also save the last integration parameters for you so all you need to do is reintegrate each sample after you do the first.

## APPENDIX D

### RAPID NITROGEN/PROTEIN ANALYSIS PROCEDURE LECO FP-528

#### EQUIPMENT:

LECO FP-528 System  
Analytical balance

#### REAGENTS:

Oxygen gas  
Helium Gas  
Air

#### PROCEDURE:

##### Instrument Start-Up:

Assumes instrument switch has been turned "ON", but gases have been turned "OFF". In the "OFF" mode, no helium is flowing.

##### QUICK MENU – First Screen

1. Perform leak detection – See operation manual for this procedure.
2. Standard parameter settings for the LECO FP528 Nitrogen/Protein System:

<u>Gases</u>	<u>Pressure</u>
Oxygen	40 psi
Air	40 psi
Helium	40 psi

When gas tanks reach 300 psi – **CHANGE TO NEW TANK**  
Combustion Tube Temperature – 850°C

•**Furnace Filter** – Change when the metal shavings have begun to rust ~1" down the tube. Change daily if the machine is used 8 hr/day.

##### •Filter Materials

Anhydrone (Mg Perchlorate) – Absorbs H<sub>2</sub>O

LecoSorb (NaOH with silica coating) – Absorbs CO<sub>2</sub>

##### •Thermal Conductivity Cell

Reference flow of Helium = 30 cc

Sample Flow = 200 cc/min – Red line is the indicator

3. To turn gases "ON"

NOTE: Superscript "S" denotes prompts on the LECO FP528 Screen while superscript "B" denotes Button below screen.

[ANALYZE]<sup>S</sup> → [I]<sup>S</sup> → [MENU]<sup>B</sup> → [5]<sup>S</sup> → [CARRIER GASES]<sup>S</sup> →  
 (ANALYZE) (SYSTEM CONTROLS)  
 [ON]<sup>B</sup> → [EXIT]<sup>B</sup>

4. To calibrate the BLANKS prior to standardization and analysis

[I]<sup>B</sup> → [SELECT]<sup>B\*</sup> → [NEXT]<sup>B\*\*</sup> → [START]<sup>B</sup> → [EXIT]<sup>B</sup> →  
 [3]<sup>S</sup> → [YES]<sup>B\*\*\*</sup> → [MENU]<sup>B</sup> → [6]<sup>S</sup> → [EXIT]<sup>B\*\*\*\*</sup> → TO QUICK  
 MENU  
 (CALIBRATE) (CALIBRATE  
 BLANK)

\*Press key two times to move to ID Code; Input Code by pressing key pad buttons until appropriate letter or number appears.

\*\*Press key to input multiple blanks >5.

\*\*\*Press to select each blank to be run.

\*\*\*\*Press 2 times.

(Allow 5 or more blanks to run until blank values are near zero (0), i.e., 0.012 or -0.012).

5. To Run Standards:

[I]<sup>S</sup> → [WEIGHT]<sup>S\*</sup> → [SELECT]<sup>B\*\*</sup> → [NEXT]<sup>B\*\*\*</sup> → [WEIGHT]<sup>S\*\*\*\*</sup>  
 →  
 (ANALYZE)

[NEXT]<sup>B\*\*\*\*\*</sup> → [ENTER REMAINDER OF STD'S] → [START]<sup>B</sup>  
 Runs Standard

\*Enter weight of standard

\*\*Press 2 times and input ID Cod, i.e. "Oats"

\*\*\*Press 1x to enter

\*\*\*\*Enter 2<sup>nd</sup> weight of standard

\*\*\*\*\*Enters 2<sup>nd</sup> standard

6. To Delete Blanks:

[PREVIOUS]<sup>B</sup> to select for DELETION  
 [NEXT]

[ANALYZE]<sup>B</sup> → [SELECT]<sup>B</sup> → (Change any blanks as needed) →  
 Scrolls through [MENU]<sup>B</sup> → [I]<sup>S</sup> DELETE  
 ID Code, Weight, P Factor



## APPENDIX E

### HUNTER LAB MINI SCAN XE PLUS STANDARD OPERATING PROCEDURES

\*\* Always handle the black and white standardization plates with care. Do not scratch or chip them.

Plug Mini Scan into electrical outlet.

Wrap PVC overwrap over aperture insuring a smooth, tight fit. Also wrap the black and white standardization plates with PVC overwrap. Make sure there are no air bubbles or wrinkles on the surface of the plates where the readings will be taken.

Wipe the black plate with a Kimwipe to insure it is clean and place the black plate on the circle of the calibration tile holder.

Place the Mini Scan on the calibration tile holder so the two rubber feet are in the two holes of the holder and the aperture is centered on the black plate. The aperture should fit flatly on the black plate to insure that there is no interference when taking readings.

Push the lightning bolt key on the Mini Scan to turn the unit on.

Make sure that the XYZ values on the screen correspond to the XYZ values listed on the back of the white plate.

You are now ready to standardize the unit. Press the lightning bolt key and the Mini Scan will read the black plate.

When the reading is complete, the screen will indicate that the machine is ready to read the white plate.

Remove the black plate from the calibration tile holder and replace it with the white plate. Wipe the white plate with a Kimwipe. Make sure that the aperture of the Mini Scan sits flatly on the white plate.

Press the lightning bolt key to read the white plate.

Press the lightning bolt key three times and the MiniScan will be ready to read the first sample.

The Tristimulus values  $L^*a^*b^*$  will be recorded.

Position the aperture of the Mini Scan on the part of the meat sample to be tested. Be sure that the aperture fits flatly on the meat but do not apply pressure. The spot to be tested should be representative of the steak **muscle** tissue. There should not be a lot of connective tissue, seam fat or subcutaneous fat where the color reading is taken.

To take a reading, press the lightning bolt key.

Record the  $L^*a^*b^*$  values and press the lightning bolt key again to take a second reading of exactly the same spot on the meat sample.

Record the  $L^*a^*b^*$  values and take a third reading by pressing the lightning bolt key.

Record the  $L^*a^*b^*$  values and press the lightning bolt key to display the average values. Record these values.

Press the lightning bolt key again to display the standard deviations. Record these values.

The Mini Scan is now ready to read the next sample. Repeat the process. Before taking readings on the second meat sample, make sure that the PVC overwrap covering the aperture is clean and free of fat or anything that might interfere with a clean reading.

When all readings are complete, unplug it from the electrical source.

Be sure that the Mini Scan is clean and that the aperture is clean before putting the machine away.

## APPENDIX F

LIPID OXIDATION ANALYSIS PROCEDURE  
FOR UNCURED MEATS**Apparatus:**

500 or 800 ml Kjeldahl flasks	400 ml beakers
Spectrophotometer with 1 cm cells	Screw cap test tubes
Hot plate or Bunsen burner	Test tube rack
Waring Blender	Graduated cylinder
Boiling chips	Timer
250 ml beakers	Pipette
Balance / Scale	

**Reagents:**

0.02 M 2-Thiobarbituric Acid (1.442 g 2-Thiobarbituric acid in 500 ml distilled water). Heat just enough to dissolve, DO NOT BOIL.

0.5% Propyl gallate (PG) and 0.5% ethylenediamine tetraacetic acid (EDTA) solution (5g PG + 5 g EDTA made up to 1 liter distilled water, heat just enough to dissolve, DO NOT BOIL).

4 N HCL (1 volume concentrated HCL and 2 volumes of distilled water) or (384 g conc. HCL in 1 liter dd-water)

Slipicone® Spray (reduces foaming)

**PROCEDURE:**

Sample/Extraction Solution Combinations for Decreasing Sample Sizes (for Step #1)

---

Meat (g)	<b>60</b>	50	40	30	20	10	2
dd-water (ml)	<b>90</b>	75	60	45	30	15	3
PG + EDTA (ml)	<b>30</b>	25	20	15	10	5	1

---

(First choice of reagents is in Bold)

1. Blend 60 g of meat with 90 ml of 50°C distilled water and 30 ml of 0.5% solution of PG and EDTA for 2 min.
2. Weight 30 g of slurry into a 250 ml beaker.
3. Quantitatively transfer beaker contents into a 500 ml Kjeldahl flask rinsing with 77.5 ml of 50°C distilled water.
4. Add 2.5 ml of 4 N HCL to the Kjeldahl flask along with 5-6 boiling chips. Spray Slipicone® into the neck of the Kjeldahl flask.
5. Turn on cooling water in the distillation unit.
6. Connect the flask to the Kjeldahl distillation unit. Turn on heat and collect 50 ml of distillate (12 – 15 min) in a graduated cylinder.



7. Remove distillate and replace with a beaker containing 400 ml of distilled water. Turn off the heat and allow water to be drawn back through the distillation apparatus. Then turn off the cooling water.
8. Add 5 ml of the distillate to a screw cap test tube along with 5 ml of the 0.02 M TBA reagent. Mix and heat in boiling water for 35 min to develop the color. For the blank, use 5 ml distilled water + 5 ml TBA reagent and heat with sample.
9. Cool in tap water for 10 min, place sample in a cuvette, then read the sample absorbance in the spectrophotometer at 530 nm. Then blank should be read first and set at 0 absorbance.

NOTE: For accurate results, a standard curve should be run for quantities of malonaldehyde over the expected range of values.

#### **CALCULATION OF TBA NUMBER:**

**TBA number = O.D. x K**

**Where K = 7.8, which was determined for the distillation set up in the lab.**

**Specifically,**

**TBA number = Abs 530 $\lambda$  x 7.8 (conversion factor) mg malonaldehyde/kg sample**

Standard deviations of the duplicates should be approximately  $\pm 0.2$  TBA Value. Slight changes occur in the K value from laboratory to laboratory. Therefore, the K value or standard curve for known dilutions of 1, 1, 3, 5 tetraethoxypropane should be calculated in each laboratory. K=7.0 is an average value that can be used but may not be the most accurate (Tarladgis et al., 1960).

**APPENDIX G****CRUDE MOISTURE AND FAT DETERMINATION – AOAC PROCEDURE****EQUIPMENT:**

Whatman® filter paper, 22 x 40 mm

Stapler

Desiccator with desiccant

Tongs

Analytical balance/ Scale

Convection oven

Soxhlet apparatus

Fume hood

Boiling chips

**REAGENTS:**

Ether (diethyl or petroleum)

**PROCEDURE:**

1. Construct thimbles from Whatman 22 x 40 mm filter paper folded into a sleeve open at one end and stapled at the other end. Dry thimbles overnight at 100°C using air dry oven. (Samples dried previously by the Air Oven method may be used.)
2. Cool thimbles in desiccator for 30 minutes.
3. Weigh thimble and record the weight (Beginning thimble weight). Put 2 to 3 grams of stirred sample into the thimble and seal. Record the weight to the nearest 0.0001 g (Beginning thimble and sample weight).
4. Dry overnight at 100°C.
5. Cool in desiccator for at least 30 minutes prior to reweighing.
6. Weigh the sample and record the weight (Dried thimble and sample weight will be used to calculate percent moisture).
7. Extract on the Soxhlet apparatus for 12 hours at an ether (diethyl or petroleum) drip rate of approximately 4 drops per second.
8. Allow sample to evaporate under the hood until thoroughly dry (no detectable ether odor) \*\* This is very important to avoid an explosion or flash fire\*\*
9. Dry in the oven overnight at 100°C.
10. Cool in the desiccator of 30 minutes or until the sample cools to room temperature (this could be as long as one hour).
11. Weigh the sample and record (Fat free thimble and sample weight).

**CALCULATIONS:**

$$\text{Percent Moisture} = \frac{100 (B-C)}{A}$$

Where: A = Sample weight  
B = Weight of dish/thimble + sample before drying  
C = Weight of dish/thimble + sample after drying

$$\text{Percent Fat Content} = \frac{(B-C)}{A} \times 100$$

Where: A = Sample Weight  
B = Dried thimble and sample weight  
C = Fat free thimble and sample weight

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