THE INFLUENCE OF ACTIVATED LACTOFERRIN AS A MICROBIAL BLOCKING AGENT ON SENSORY AND SHELF LIFE CHARACTERISTICS OF CASE-READY FRESH BEEF

By

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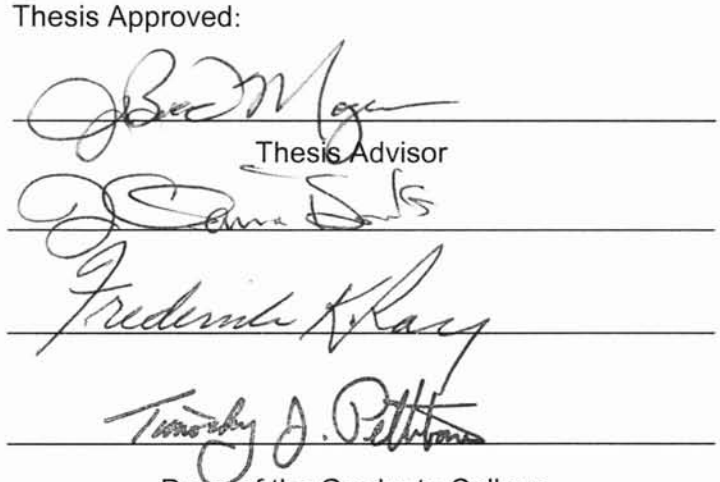

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FORMAT OF THESIS

This Thesis is presented in the Journal of Animal Science style format, as outlined by the Oklahoma State University graduate college style manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

CHAPTER I

INTRODUCTION

The beef industry and its products are endlessly scrutinized by the media. It is such publicity that has developed the current image of beef with regard to food safety. In 2001, 57 % of the beef industry's media coverage was regarding beef safety issues (National Cattleman's Beef Association (NCBA), 2002). In the eyes of many consumers, beef has developed the reputation of being prone to post-harvest contamination, even though it has been proven that the U.S. generates the safest beef supply in the world (Morgan et. al., 1995).

Attainment of safe food incorporates all conditions and measures that are necessary during production, processing, storage, distribution, and preparation to ensure a safe, sound, wholesome product fit for human consumption. The matter of food safety and the threat of contamination to the United States beef supply is real and must be addressed. It is improbable that such a challenge disappears; thus, demands industry support through continuous research and external funding. The NCBA (2002) has been a leading contributor since 1993 allocating more than $7 million towards food safety research.

Due to various reasons, including food safety concerns, the United States beef industry has been losing market share to the pork and poultry industries since 1976 (United States Department of Agriculture (USDA), 1999). In 1992, per

capita consumption of chicken surpassed beef. Total meat consumption has been relatively constant for the past 20 years, and as the consumption of beef was on the decline, the consumption of poultry was on the rise (USDA, 1999). Innovative meat product development that meets consumer demands, case-ready technology, product consistency, and marketing have lead the poultry industry to the forefront of protein sales.

The red meat industry, particularly the beef sector, has finally realized that borrowing the poultry industry's technology was much more effective than competing with them. As a result, the beef industry has inverted their demand curve, and in 10 of the last 12 quarters beef demand increased (NCBA, 2002). The recent merger of the leading poultry supplier with the largest pork and second largest beef supplier has jump-started the revolution of the meat industry in the early years of the twenty-first century.

As the struggle for lost market share continues, researchers pursue new technologies to improve the safety and the image of the beef industry. Technology such as microbial blocking agents (MBA) are currently under review and hold great promise. Presently, numerous antimicrobial agents, heat-treatment processes (pasteurization), washing techniques incorporating organic acids, and innovative packaging materials are being employed by the meat industry as intervention steps in the food safety chain.

As live animal and fresh meat producers attempt to meet the demands of today's consumer-driven industry, end-users must realize that partial food safety responsibility falls onto their own shoulders. Consumers rely heavily on others

for the consumption of safe food and prefer not to accept their role in food safety (Doores, 1999). Smith (2000) disturbingly reported that 9% of shoppers take more than two hours after leaving the grocery store to refrigerate or freeze beef cuts. Food safety incorporates all conditions, including handling and preparation of foods after purchase.

Consumers are more aware and concerned about the wholesomeness of their food. And it seems they should be, as the incidence of foodborne illness is on the rise. However, it is difficult to decipher if foodborne illness is truly on the rise or if current methods for detecting and evaluating foodborne disease are more efficient (Doores, 1999). Foodborne illnesses as defined by the World Health Organization (WHO) (2000) are diseases caused by agents that enter the body through the ingestion of food. *Escherichia coli* O157:H7 (*E. coli* O157:H7) is a pathogen that has plagued the beef industry and has emerged as a major public health concern in the United States (Padhye and Doyle, 1991). The WHO (2000), estimates that there are 76 million cases of foodborne disease resulting in 325,000 hospitalizations and approximately 5,000 deaths annually in the U.S. alone. Additionally, the WHO predicts an annual loss of productivity and medical costs resulting from major foodborne pathogens in the U.S. approaching $37.1 billion. NCBA (2002) reported that in the past five years over $12 million has been spent on food safety research. This resulted in 99.5 % and 99.7 % reductions in *E. coli* occurrence counts and microbial plate counts on U. S. beef carcasses, respectively (NCBA, 2002).

The United States Department of Agriculture has established a zero-tolerance policy for *E. coli* in ground beef and on beef carcasses, requiring "removal of all soil (ingesta, milk, feces) contamination prior to washing and chilling of carcasses" (Horne 1993; Smith and Sofos, 1994). Cooking parameters are known which can free contaminated meat of *E. coli* O157:H7. However, cattle have been implicated as a principal reservoir of *E. coli* O157:H7 (Faith et al., 1996) and, cross-contamination is a viable threat. Consequently, the complete and total elimination of pathogens in the United States beef supply is an improbable occurrence. Nevertheless, a pathogen free, raw, red meat product is the optimal objective; one that consumers expect. Despite bovine spongiform encephalopathy (BSE) and foot and mouth disease outside the United States, consumer confidence has remained strong. As of July 2002, NCBA reported 86 % of consumers are confident that U.S. beef is safe.

Considering the current objective to improve food safety and rejuvenate the image of beef, researchers are open to any prospective venue. Microbial blocking agents (MBA) are one of the most recent food safety techniques available. Lactoferrin (LF), a heat stable protein found in biological fluids such as milk, (Johansson, 1960; Bullen et. al., 1972) can be classified as a MBA. Lactoferrin protein has been studied and utilized in the medical field for years. However, recently was introduced to the red meat industry as a possible MBA. Lactoferrin has been reported to have antimicrobial activity against many of the problematic microorganisms associated with fresh beef, including a broad range of gram-positive bacteria, gram-negative bacteria (Arnold et. al., 1977; Bortner et.

al, 1986; Kalmar and Arnold, 1988) including *E. coli* O157:H7 (Jones et. al., 1994), fungi and protozoa (Naidu and Bidlack, 1998). The amount of lactoferrin required to be effective is thousands of times less than the amount in a single glass of milk (Fremont, 2001). The antimicrobial activity of LF was originally attributed to its ability to sequester two atoms of iron (Oram and Reiter, 1961; National Institute of Child Health and Human Development, 1994), an essential bacterial nutrient (Chapple et al., 1998), for every one molecule of LF. However, recent discovery of a LF peptide, which is dislocated from the iron binding sites, provides strong evidence of a bactericidal mechanism that is independent of iron (Dionysius and Milne, 1997).

Due to LF's activity as a MBA, researchers speculate that LF can form a barrier protecting fresh meat from bacteria present as well as future contamination. The objective of this research was to evaluate the ability of activated LF to increase shelf life as a result of reduced total plate counts while retaining desirable organoleptic characteristics of case-ready fresh beef strip loin steaks.

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CHAPTER II

REVIEW OF LITERATURE

Food Safety Perceptions

It is estimated that about twenty percent of the world's food supply is lost due to microbial spoilage (Branen, 1983). Spoilage and contamination of fresh meat products have been combated with antimicrobial agents, both natural and chemical, as well as packaging techniques such as modified atmosphere packaging (MAP). Antimicrobials not only serve to provide the food safety desired by the consumer, but also to increase product shelf life by preventing food spoilage (Naidu and Bidlack, 1998). There are numerous antimicrobial agents that are currently utilized in the red meat industry such as benzoic acid, nitrites, sulfites, vitamin E and rosemary. Increasingly, naturally occurring agents have gained attention because of the demand for 'all natural' food products (Beuchat and Golden, 1989).

Lactoferrin can be classified as a natural microbial blocking agent (MBA) that occurs in biological substances, primarily milk, and has the ability to inhibit growth-multiplication of microorganisms (Naidu and Bidlack, 1998). This protein has been studied and utilized in the medical field for years. However, recently was introduced to the red meat industry as a possible antimicrobial agent.

Prospective preservatives for the use in fresh meat must cater to the following criteria:

- non-toxic product that can be utilized on animals and humans
- metabolized and excreted
- water soluble, media of microbial growth
- heat stable to with stand thermal processes
- active over a wide pH range

Lactoferrin, isolated from bovine milk whey, conveniently possess all of the above listed qualities.

Lactoferrin: Background

The majority of the founding research on lactoferrin was conducted in the medical community. Extensive work by Masson and coworkers (1966, 1968, 1969) has established a clear role for lactoferrin in cellular immunity. Unlimited research has been completed and published that supports the immuno-functional properties of LF in human health. Some of the early work of Bullen and associates (1972) stated that breast-fed infants are better protected against disease than infants fed commercial formulation. In support of medical research, Sanchez and coworkers (1988) reported LF concentrations of 1.2 mg/mL in milk from mastic cows compared to 0.09 mg/mL in milk from healthy cows.

Upon detection of LF in natural biological systems, researchers began to develop methods of isolation. Lactoferrin was first isolated from bovine milk

whey, the yellow-green liquid that separates from the curd during the manufacture of cheese and casein, which has long been considered by the dairy industry as a waste by-product (Smithers et. al., 1996). Riedel (1994) reported worldwide annual production of liquid whey reaching 118 million tons with 7 million tons of whey solids. It is estimated that less that 62% worldwide whey production is gainfully utilized. The major component proteins of whey have an isoelectric point (pI) of less than 7.0; however, the pI of LF protein is greater than 9.0. Therefore, cation-exchange column chromatography at neutral pH is the most commonly reported method for LF extraction from whey (Law and Reiter, 1977). The percentage recovery of LF can be as high as 62% from whey that has undergone mild processing. However, it can be as low as 12% from whey subjected to high heat treatment and mechanical damage (Smithers et. al., 1996). Researchers have developed and applied assays for protein isolation, which allow for greater volumes and higher flow rates.

Following successful isolation, a revolutionary facet of LF research began. Today LF is an additive in such items as infant formula, sports drinks, functional foods, personal care products, supplemental tablets, veterinary supplies, and animal feed specialties.

Lactoferrin: Chemical and Physical Properties

Lactoferrin is an iron scavenging single-chained, glyco-protein of the transferrin family (Masson et. al., 1969; Naidu, 2000a). It is present in most exocrine secretions of mammals including milk and colostrum (Johansson, 1960;

Masson et. al., 1969; Reiter, 1983). Lactoferrin levels in bovine colostrum range from 2.0 to 5.0 mg/ml and in mature bovine milk range from 0.1 to 0.3 mg/ml (Bishop et. al., 1976). Lactoferrin can also be found in saliva, tears, and seminal fluids.

Table 1: Sources and Amounts of Lactoferrin Present in Biological Fluids. (DMV International, 2001).

Biological Fluid	**Amounts**
Colostral breast milk	7 mg/ml
Mature breast milk	1-2 mg/ml
Tear fluid	2.2 mg/ml
Seminal plasma	0.4-1.9 mg/ml
Synovial fluid	0.01-0.18 mg/ml
Saliva	0.007-0.01 mg/ml
Colostral cow milk	2.0-5.0 mg/ml
Mature cow milk	0.02-0.3 mg/ml

Functionally, lactoferrin has the following biological attributes:

1) the ability to reversibly bind a variety of metals with high affinity,
2) the ability to bind cations, and
3) the ability to bind numerous types of biological cells.

A single molecule of LF has the capacity to reversibly bind 2 ferric iron (Fe^{+3}) ions with high affinity ($K_a = 10^{20}$ L/mol) (Naidu and Bidlack, 1998), an essential bacterial nutrient, (Chapple et. al., 1998) in the presence of carbonate (CO_3^{2-}) or bicarbonate (HCO_3^-) giving rise to the possibility of antimicrobial

activity. However, recent research indicates that a LF peptide, lactoferricin (Lfcin), exhibits bactericidal activity, which is iron independent.

Isolated bovine lactoferrin (LF) displays a metal binding mechanism with the following features:

1) synergistic relationship between cation and anion,
2) extremely tight bind with iron (cation), and
3) ability to release tight bond.

Other metal cations of similar size can be substituted for iron (Ga, Al, Cr, Mn, Co) with reduced bind affinity (National Institute of Health (NIH), 1994).

Lactoferrin: Mode of Action

The antimicrobial mechanism of LF has been postulated by many researchers. However, LF is a complex molecule with numerous potential functional properties. Therefore, the definite mode of action is uncertain. In general, antimicrobial agents are substances which inhibit growth or destroy microorganisms (Madigan et. al., 1997). Antimicrobial activity can result in a static effect (inhibition of growth, but not death) or a cidal effect (death of organism).

Naturally occurring LF derived from bovine milk, as well as other biological secretions, is virtually iron free (apo-LF) with less than 10 % iron saturation. Apo-LF has exhibited pronounce bacteriostatic properties in vitro; likely due to iron chelation (NIH, 1994). Iron saturated LF is referred to as holo-LF and is less active. Researchers identified a bactericidal domain (amino acid sequence)

localized in the N-terminus of LF, which does not contain iron-binding sites (Bellamy et. al., 1992b; Tomita et. al., 1994; Dionysius and Milne, 1997). The following table illustrates the antimicrobial spectrum of LF and LFcin as well as the mechanism by which organism are effected.

Table 2: Inhibitory Spectrum of Bovine Lactoferrin (LF) and Lactoferricin (Lfcin) against various bacteria (Naidu, 2000a).

Bacterial Species	Form	Dose	Effect	Reference
Aeromonas hydrophila	LF	0.1%	Adhesion-blockade (47%)	Paulsson et. al., 1993
Bacillus cereus	LFcin	6 µM	Cidal (4-log, 100%)	Hoek et. al., 1997
Bacillus circulans	LFcin	0.006%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Bacillus natto IFO3009	LFcin	0.002%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Bacillus stearothermophilus	LF	1:20	Stasis	Reiter & Oram 1967
Bacillus subtilis	LF	1:20	Stasis	Reiter & Oram 1967
Bacillus subtillis ATCC6633	LFcin	0.002%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Bifidobacterium longum	LF	0.1%	Agglutination	Tomita et. al., 1994
Corynebacterium diphtheriae	LFcin	0.018%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Corynebacterium ammaniagenes	LFcin	0.003%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Corynebacterium renale	LFcin	0.001%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Clostridium innocuum	LF	0.1%	Agglutination	Tomita et. al., 1994
Clostridium perfringens	LFcin	0.024%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Clostridium paraputrificum	LFcin	0.003%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Enterococcus faecalis	LFcin	0.06%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Escherichia coli E386	LF	0.1%	Stasis (24-h 100%)	Naidu et. al., 1993
Escherichia coli H10407	LF	0.1%	Adhesion-blockade (50%)	Paulsson et. al., 1993
Escherichia coli IID-861	LFcin	10 µM	Cidal (3-log reduction)	Bellamy et. al., 1992b
Escherichia coli CL99	LF	20 µM	LPS release, OM damage	Yamauchi et. al., 1993
Escherichia coli O157:H7	LFcin	15.6 mg	Cidal (99.9 %)	Jones et. al., 1994
Klebsiella pneumoniae	LFcin	10 µM	Cidal (3-log reduction)	Bellamy et. al., 1992b
Lactobacillus casei	LFcin	0.01%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Listeris monocytogenes	LFcin	10 µM	Cidal (4-log reduction)	Bellamy et. al., 1992b
Listeris monocytogenes NCTC7073	LFcin	2 µM	Cidal (4-log, 100%)	Hoek et. al., 1997
Micrococcus luteus	LF	0.1%	Agglutination	Tomita et. al., 1994
Proteus vulgarus JCM1668T	LFcin	0.01%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Pseudomonas aeruginosa IFO3446	LFcin	10 µM	Cidal (4-log reduction)	Bellamy et. al., 1992b
Pseudomonas fluorescens	LFcin	8 µM	Cidal (4-log, 100%)	Hoek et. al., 1997
Salmonella abony	LF	0.8%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Salmonella Dublin	LF	0.2%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Salmonella enteritidis	LFcin	0.01%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Salmonella Hartford	LF	0.8%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Salmonella Kentucky	LF	0.2%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Salmonella panama	LF	0.1%	Stasis (24-h 100%)	Naidu & Arnold, 1994

Bacterial Species	Form	Dose	Effect	Reference
Salmonella pullorum	LF	0.2%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Salmonella rostock	LF	0.2%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Salmonella salford	LFcin	4 μM	Cidal (4-log, 100%)	Hoek et. al., 1997
Salmonella Montevideo	LF	20 μM	LPS release, OM damage	Yamauchi et. al., 1993
Salmonella Thompson	LF	0.1%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Salmonella typhimurium Rd	LF	0.5%	Stasis (64%)	Naidu et. al., 1993
Salm. Typhimurium R10	LF	0.1%	Adhesion-blockade (68%)	Paulsson et. al., 1993
Salm. Typhimurium SL696	LF	20 μM	LPS release, OM damage	Yamauchi et. al., 1993
Salmonella virchow	LF	0.8%	Stasis (24-h 100%)	Naidu & Arnold, 1994
Shigella flexeri	LF	0.1%	Adhesion-blockade (30%)	Paulsson et. al., 1993
Staphylococcus albus	LF	0.5%	Stasis	Masson et. al., 1966
Staphylococcus aureus	LF	0.1%	Adhesion-blockade (54%)	Paulsson et. al., 1993
Staphylococcus aureus JCM2151	LFcin	10 μM	Cidal (3-log reduction)	Bellamy et. al., 1992b
Staphylococcus epidermidis	LFcin	0.006%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Staphylococcus haemolyticus	LFcin	0.001%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Staphylococcus hominis	LFcin	0.003%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Streptococcus bovis	LFcin	0.006%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Streptococcus cremoris	LFcin	0.003%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Streptococcus lactis	LFcin	0.003%	Cidal (6-log, 100%)	Bellamy et. al., 1992b
Streptococcus thermophilus	LFcin	0.003%	Cidal (6-log, 100%)	Bellamy et. al., 1992b

Microbial Blocking Agent. Microbial blocking agent is a new term applied to a class of naturally occurring innate defense factors of the mucosa that block microbial adhesion-colonization and growth multiplication (Naidu, 2000b). Microbial blocking agents, unlike microbicidals, can inactivate and/or scavenge endotoxins (lipopolysacchrides), cytotoxins, pro-inflammatory substances, and residual DNA of the microbial debris from the milieu (Naidu and Bidlack, 1998). Lactoferrin's extreme affinity to a bacteria-essential nutrient, iron, acts as an adhesion blockade and causes organism starvation. Bovine LF has been shown to have bacteriostatic activity against a broad range of gram-positive bacteria, gram-negative bacteria (Arnold et. al., 1977; Bortner et. al, 1986; Kalmar and Arnold, 1988) including *E. coli* O157:H7 (Jones et. al., 1994), fungi and protozoa

(Naidu and Bidlack, 1998). Additionally, the inhibition of adhesion, colonization, and growth-multiplication (stasis effect, not cidal effect) of *Bacillus* sp. (gram negative bacteria) was reported, associating the cause to be deprivation of the bacteria from essential iron (Oram & Reiter, 1961). Lactoferrin has notably shown a stasis effect against other prevalent foodborne illness related pathogens such as Salmonella, Camphylobacter and *E. coli* O157:H7 (Naidu, 2000a).

Cation Effect. Gastric pepsin cleavage of LF produces a 25 amino acid peptide, lactoferricin B (Bellamy et. al., 1992b; Naidu, 2000a). Lactoferricin B exhibits broad-spectrum antibacterial activity, with the inhibition of gram positive and gram-negative bacteria, including strains resistant to native LF (Naidu, 2000a). Hydrolysis of bovine LF by porcine pepsin, cod pepsin of acid protease from *Penicillium duponti* results in low molecular weight peptides with strong activity against *E. coli* 0111, where hydrolysis by trypsin, papain, and other natural proteases results in less active peptides (Tomita et. al., 1991).

The proposed mechanism of LF as a bactericide is as a cationic chelator. The LF peptide domain responsible for cidal activity is dislocated from iron binding sites and is attributed to a specific amino acid sequence, which has many basic and hydrophobic amino acid residues. Any LF peptide containing this sequence can demonstrate bactericidal activity; however, effectiveness varies with amino acid chain length and hydrolysis method. Lactoferrin peptides containing the cidal domain sequester cationic biological cofactors, which are involved in bacterial membrane permeability (Westerhoff et. al., 1989; Hill et. al., 1991; Naidu, 2000a). The iron binding protein peptides can affect gram-negative

outer membranes in a similar manner to the chelator ethylenediamenetetraacetic acid (EDTA) (Ellison et. al., 1988). EDTA is commonly known as a blood anticoagulant in which a congruent mode of action binds clotting ions present; thus, preventing clot formation. The LFcin B peptide directly damages the cell structure and affects the permeability of gram-negative bacteria outer cell membranes (cidal effect) (Ellison, et. al., 1988; Yamauchi et. al., 1993). Loss of bacterial viability can be observed after only 10 minutes of exposure to LFcin B. The rate of killing can be is consistent with the rate of binding with the bacterial cells. Direct interaction of LFcin B with bacterial cell surface is necessary for a lethal effect (Bellamy, et. al., 1993). Lysis of cells and death of bacteria is a probable result. Native bovine LF does not exhibit a cidal effect (Naidu, 2000a); only LF peptide derivatives. The explanation regarding native LF's lack of cidal activity is not fully understood.

Muscle Chemistry: Oxidation

Two basic categories of oxidation exist; that which occurs during storage and that which occurs rapidly upon cooking (Pearson and Young, 1989). Oxidation during storage of fresh meat will be the topic of this section as it is of primary concern relative to shelf life.

There are numerous catalysts which promote the oxidation of both muscle pigments and lipids; thus influence ultimate shelf life. Extrinsic factors such as lighting (photoxidation), gaseous atmosphere in packaging system, temperature, and hygiene (microorganisms) in conjunction with intrinsic factors such as pH,

water activity (a_w), and O_2 reduction potential all play a key role in the oxidation rate of fresh meat.

Oxygen in excess of what is required for the progression of deoxymyoglobin (DMb) to oxymyoglobin (OMb) can be reduced via a one-electron reduction process to free radicals:

1. Hydroxyl radical ($HO^{\cdot}$)
2. Hydrogen peroxide (H_2O_2)
3. Perhydroxyl radical ($HO_2^{\cdot}$)
4. Superoxide anion radical ($O_2^{\cdot}$)

These oxygen-derived radicals have the ability to initiate lipid and pigment oxidation. Oxygen is an unusual diatomic molecule, which has two unpaired electrons forming a triplet ground state. Singlet oxygen is formed when triplet oxygen absorbs sufficient energy to shift one of the unpaired ground state electrons to a higher energy level. Singlet Oxygen, in an unstable energy state, releases excess energy by reacting with electron rich double bonds such as those found in polyunsaturated fatty acids (PUFA) (St. Angelo, 1996).

Pigment Oxidation and Color. Color is the single most influential factor affecting consumer perception and purchase intent of fresh meat (Mackinney et. al., 1966; Greene et. al., 1971; Sherbeck et. al., 1995). The bright cherry red color typically associated with freshness of beef is a result of two predominate respiratory pigments, myoglobin and hemoglobin, which are present in muscle ante- and post-mortem. Myoglobin and hemoglobin can be considered heme proteins; large proteins consisting of a porphyrin ring containing a central iron

atom. Hemoglobin is the primary pigment in blood, which functions as an oxygen transporter. The hemoglobin complex in living organisms is purple until exposed to oxygen (O_2). Myoglobin is a quarter of the size of hemoglobin (Pearson and Young, 1989) and is located within muscle fibers. Due to the iron atom, myoglobin displays a similar color change in the presence of O_2. In well-bled muscle tissue myoglobin constitutes 80 to 90 % of the total pigment (Hedrick et. al., 1994).

The ultimate color of muscle is largely determined by the chemical state of myoglobin; specifically, the oxidation state of the iron atom (Cross et. al., 1986). If iron is oxidized (ferric, Fe^{3+}) it cannot bind other molecules. However, if the iron atom is reduced (ferrous, Fe^{2+}) it can readily bind with water (in unexposed muscle) and oxygen (muscle exposed to the air). There are three naturally occurring pigments formed by myoglobin; deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb) (Hedrick et. al., 1994; Kanner, 1994).

In living tissues, reduction occurs naturally via the electron transport chain. Deoxymyoglobin, the reduced form of myoglobin with iron in the ferrous state (Fe^{2+}), is present in living tissue as well as unexposed muscle and results in dark purplish-red pigment (Hedrick et. al., 1994). In the presence of atmospheric air, approximately 20 % O_2 (Brown et. al., 1994), deoxymyoglobin spontaneously forms oxymyoglobin and produces what is typically thought of as fresh beef color, bright cherry-red (Hedrick et. al., 1994; Cross et. al., 1986). Although this reaction is spontaneous, it is not instantaneous. The reaction of deoxymyoglobin with O_2 to form oxymyoglobin requires approximately 30 minutes. Development

of oxymyoglobin and a bright cherry-red color is known as *bloom time* (Smith et. al., 1996). This reaction is also highly affected by the oxygen consumption rate (OCR). As atmospheric pressure increases, oxygen penetration into muscle interior increases (Schuler, 1990). The ultimate depth of O_2 penetration and subsequent OMb formation results in what is referred to as the met-line (M-TEK, 1998); where the OMb ends and MMB begins. The appearance of discoloration diminishes with greater oxygen concentration and penetration (Daun et. al., 1971) due to quantity and oxidation rate of OMb. It is this scientific theory that has given rise to the advent of high oxygen case-ready packaging systems.

Oxymyoglobin is relatively stable and not easily oxidized to metmyoglobin under normal atmospheric conditions. However, if deoxymyoglobin is exposed to small quantities of O_2 rather than atmospheric air, such as in low oxygen packaging systems without O_2 scavengers, deoxymyoglobin can oxidize to form metmyoglobin, which results in brown pigment. Metmyoglobin and the appearance of pigment discoloration remain in muscle pigment oxidized from DMb while in the presence of atmospheric air. Reduction to a desirable pigment only occurs when oxygen is completely eliminated from the system (Hedrick et. al., 1994). This is a serious marketing concern for the beef industry because consumers associate brown meat with product that is unwholesome (Greene et. al., 1971; Sherbeck et. al., 1995); whereas, this specific reaction alters pigmentation; microbial soundness remains unaffected.

Metmyoglobin can also be derived from oxymyoglobin, which begins upon oxygenation. However, because OMb is relatively stable, this oxidation reaction progresses slowly. When the percentage of oxymyoglobin (Fe^{2+}) that is oxidized to metmyoglobin (Fe^{3+}) reaches 40 to 60 % muscle tissue will begin to exhibit characteristics of pigment discoloration and appear brown (Lawrie, 1985).

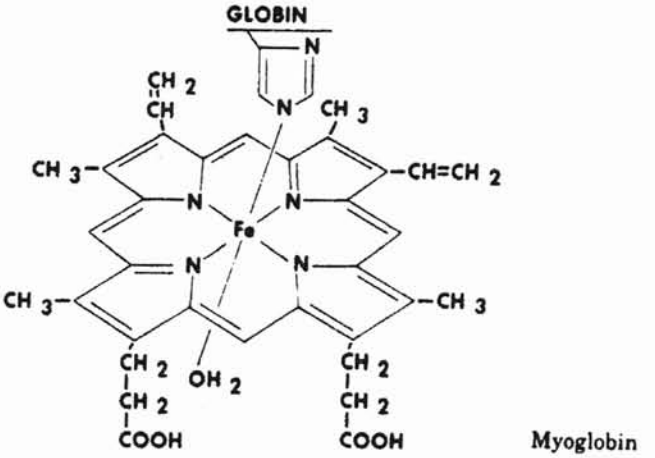

Figure 1: Chemical Structure of Myoglobin with Diagram of Central Iron Atom Oxidation State and Group Occupying the Sixth Bond Orbital (Pearson and Young 1989).

The metal compounds (i.e. iron) of myoglobin and hemoglobin can be activated and oxidized from the ferrous (DMb and OMb) to the ferric (MMb) state. Hydrogen peroxide (H_2O_2) has been identified as the primary initiator and is highly correlated with the discoloration of beef (Pearson et. al., 1977; Kanner, 1994). Additionally, the rate of oxidation in OMb and DMb is increased by temperature abuse (George and Stratmann, 1952; Snyder and Ayres, 1961; Walters, 1974). In support, Ramsbottom and Koonz (1941) reported that low storage temperatures depressed enzyme activity, minimized color changes, inhibited oxidation and reduced desiccation and drip. Similarly, a study conducted by Hood (1980) which evaluated temperature effects on prepackaged beef after 96 hours of shelf life at varying temperatures (0, 5, and 10°C) concluded that increased temperature resulted in significantly higher ($P< 0.0001$) rates of muscle discoloration.

The relationship of meat color and pH has been researched extensively, whereas, the effect of pH on oxidation is controversial. Conclusive findings are that meat with an ultimate pH approaching the isoelectric point (pI) of actomyosin (5.0) lose electrostatic repulsion; thus, have decreased water holding capacity, excess extracellular water (exudative) and a washed-out, pale appearance. In contrast, meat with a high ultimate pH maintain the majority of water as intracellular water, exhibit increased water holding capacity, and appear to be a dark red (Hedrick et. al., 1994; Byrem and Strasburg, 2000). George and Stratmann (1954) reported that at a low pH there was an accelerated product discoloration. In contrast, Hood (1980) published that pH had no significant

effect on the rate of oxidation and discoloration. It is crucial to note that Hood's study utilized product with a limited pH range, which could possibly explain the conflicting results.

Pigment color relative to shelf life is highly dependent on the oxidation reaction involving OMb and DMb. The reaction rate fluctuates, as do all biological reactions, on account of available reducing equivalents ($NADH_2$) and metmyoglobin reducing activity (MRA) (Cross et. al., 1986). The retention of myoglobin in the reduced state (i.e. OMb) of retail beef results in optimal shelf life and the preservation of bright cherry-red color (Smith et. al., 1996).

Lipid Peroxidation. Lipid and pigment oxidation are coupled; however, not fully understood (Greene, 1969; Faustman et. al., 1989). What can be stated for certain is that oxidation of fatty acids in animal tissue begins almost immediately postmortem (Gray and Pearson, 1994). Most researchers concur that lipid oxidation, the combination of organic compounds with atmospheric oxygen, proceeds via chain reactions involving peroxy radicals (Gray, 1978; Allen and Hamilton, 1983; St. Angelo, 1996). It is the origin of the free radical that is unknown. Initiation of autoxidation can be activated by any of the previously mentioned radicals, as well as temperature (heat), and radiation. Polyunsaturated fatty acids are the most susceptible lipid substrate to oxidation, as they are least stable containing the most double bonds (Gray, 1978; Allen and Allen, 1981). The initial step generates hydroperoxides that are unstable and decompose with the loss of a hydrogen atom to produce free lipid radicals. Lipid free radicals rapidly react with oxygen to form peroxyradicals. The generation of

peroxyradicals completes the chain reaction and allows for continuation; each peroxyradical scavenges hydrogen atoms from surrounding hydrocarbon chains producing new peroxyradicals to continue autoxidation (i.e. self initiation) and the free radical chain reaction. The cumulative reaction of lipid oxidation results in secondary products such as aldehydes, acids, and ketones; all of which are responsible for the development of off-flavors and odors associated with oxidative rancidity in meat (Hedrick et. al., 1994; Shahidi, 1994; St. Angelo, 1996).

The rate of lipid autoxidation is increased by several pro-oxidants such as heat, low pH, metal ions, ultraviolet light, and sodium chloride (Hedrick et. al., 1994). Watts (1954) reported that oxidation rates double with every 10°C increase in temperature. In order to extend the shelf life, retard oxidative rancidity and reduce discoloration of fresh meat, pro-oxidants must be recognized and accommodated with low storage temperatures, reduction of oxygen in package atmosphere, the elimination of light, and possible incorporation of synthetic and natural antioxidants such as α-tocopherol and rosemary.

Retail Merchandising: Case-Ready

The case-ready evolution can be typified by a National Cattlemen's Beef Association quote.

"The advent of case-ready product, deemed the most significant advance in beef processing since the advent of boxed beef in the late 1960's, has already reshaped the way beef is processed, packaged, and marketed to consumers" (National Cattleman's Beef Association, 2000).

Traditional retail fresh meat cases are outdated, being readily replaced by a case-ready system in which products arrive at retail stores prepackaged and often pre-priced. Prepackaging in the fresh meat business typically refers to modified atmosphere packaging (MAP). Modified atmosphere packaging can be defined as the packaging of a perishable product in atmosphere modified so that its composition is other than air (Hintlian and Hotchkiss, 1986). A U.S. survey conducted by Cryovac Division of Sealed Air Corporation (2001) in January 2001 revealed that of the 127,000 grocery stores in the U.S., 25,000 offered case-ready poultry, 10,000 offered case-ready beef, 6,000 offered case-ready pork, and 1,000 offered case-ready total muscle cuts. Furthermore, an average growth in sales of 3.8 % is estimated for stores converting to case-ready marketing. Similarly, the American Meat Institute (2001) reported that in 2000 retailers were selling 1.2 billion case-ready meat packages, more than double that sold in 1997. This trend progressed as a result of the many advantages offered by case-ready meat systems.

1. Increased Profitability:
    - Reduced shrinkage
    - Discarding, reworking, discounting is reduced/eliminated
    - "Just in Time" delivery systems reduces inventory
    - Provides beef merchandisers an opportunity to "sell" beef items
2. Enhanced Safety:
    - Centralized fabrication system which produces uniform, consistent product
    - Lower microbial contamination while lengthening retail shelf life

- Increased quality control as product leaves federally inspected plant in a sealed package that isn't opened until the consumer takes it home

3. Value-Added Offering:
    - Consistent supply of entire beef offering
    - Allows offering of specialty cuts/orders

In regard to food safety, the elimination of product handling within retail outlets is key. By containing products before they leave a federally inspected plant, merchandisers can decrease the opportunity for contamination and increase shelf life, while removing responsibility from the retail store chain. Case-ready also allows for the transfer of labor from the retailer to the packing plant; thus, reducing cost (repackaging), the need for skilled labor (butchers), and increasing the availability of in-store space. An important aspect of a retail meat case is supplying product that is in demand, particularly seasonal and holiday items. Case-ready packaging systems give retailers this ability; to keep a full case of in-demand product. The reduction of product variability is naturally resolved by replacing individual butchers with mass production by only a few processors. Conveniently, large processors have the capability to pre-weigh and pre-price products allowing for easier trace-ability and quantifiable sales. For years red meat products were one of the few food items without brand names. Recently, as a result of case-ready marketing, companies have the ability to develop brand name customer loyalty. Customers can expect to receive the same product from every store throughout an individual chain.

Along with the beneficial aspects of case-ready packaging come disadvantages; primarily, distribution obstacles. In the past, the majority of fresh meat products were transported as subprimals via vacuum packages containing no excess headspace. Vacuum bags were opened at the retail store and subprimals were fabricated into retail items (i.e. steaks, roasts), which were repackaged in trays and overwrap. Overwrap packaging when compared to case-ready modified atmosphere packaging results in decreased shelf life and increased product shrink. Shelf life can be defined as the amount of time a fresh meat product remains acceptable during retail display prior to discounting or removal from the case. Optimizing the shelf life of fresh meat products is essential; decreased product loss results in increased revenue. However, in return for increased shelf life industry has incurred the cost of transporting what used to be two boxes of vacuum packaged product to transporting 4 to 5 boxes of MAP product. The means to minimize distribution cost ultimately translates to profit. Centralized packaging facilities are being added as a tool to decrease these incurred costs.

Retail Merchandising: Packaging Technology

Freshness of beef is associated with a bright, cherry red lean color (Shay and Egan, 1987; Hunt, 2002); a result not directly due to freshness, but to oxygenated myoglobin. Immediately after oxygenation (i.e., "blooming"), oxymyoglobin begins to oxidize into MMb (brown pigment). Products are

typically discounted at the retail level when metmyoglobin percentages approach 70% (Lawrie, 1985).

Obviously, the factor retarding the advancement of beef into case-ready products is color stability. Employment of antioxidants, antimicrobials, and various gaseous atmosphere combinations are used to combat retail case discoloration. There are three basic forms of packaging for fresh meat: air (overwrap and trays), vacuum, and modified atmosphere. Numerous types of MAP exist, which utilize combinations of oxygen, nitrogen (N) and carbon dioxide (CO_2) along with various films, bags, trays, and scavengers.

The gas involved in MAP plays a critical role in the ultimate shelf life of fresh meat products. Oxygen is vital in the color development of fresh red meat. Without O_2, beef would not appear bright cherry-red. A higher concentration of O_2 in a package atmosphere decreases the met-line by inducing a deeper layer of oxymyoglobin (Daun et. al., 1971); thus, extending color stability and shelf life (Hunt, 2002). Carbon dioxide functions very effectively as an antimicrobial by increasing the microorganism's lag phase and reducing respiration (Tewari et. al., 1999). Nitrogen, the primary constituent in the earth's atmosphere, is utilized in MAP to displace O_2 and act as a filler. Nitrogen will prevent package collapse upon CO_2 absorption by moisture in the product (Tewari et. al., 1999).

The development of modified atmosphere packaging began in 1922 when Brown (1922) analyzed the effects of O_2 and CO_2 on the germination and growth of fruit-rotting fungi. Research conducted by Killefer (1930) showed two times the shelf life of fresh pork and lamb packaged in 100 % CO_2 stored at 4-7°C

compared to its counterpart stored in air. Similarly, Blickstad and coworkers (1981) reported a 40 d shelf life of product packaged in pure CO_2 compared to 10 d of shelf life for control product packaged in air. As early as 1951 researchers stated that the shelf life of fresh meat was a linear function of CO_2 concentration (Ogilvy and Ayres, 1951) and that as little as 4% CO_2 can retard mold growth on meat (Moran et. al., 1932; Tomkins, 1932). Haines (1933) observed that multiplication of common bacteria to a certain endpoint took twice as long in an atmosphere of 10 % CO_2 at 0°C than in normal air. During the 1970's, bulk packages of chicken flushed with CO_2 were commercialized in the US with an 18 to 21 day shelf life in refrigerated storage (Parry, 1993). Despite the benefits, atmospheres containing high CO_2 concentrations failed to retain product quality (Ogilvy and Ayres, 1951) often resulting in dehydration and discoloration amidst low microbial loads.

Vacuum packaging and the benefits of anaerobic storage conditions were simultaneously being research. Vacuum packaged fresh meat is stable for approximately 3 to 4 wks providing temperature controls close to 0°C (Labadie, 1999) due to the limited number of microorganism that have the capability to grow in anaerobic conditions at refrigeration temperatures. Simplicity and extended shelf life rapidly advanced the acceptance of vacuum package technology and was popular by the 1960's. This technology is highly effective as well as economical. Consequently, vacuum packaging is still utilized for processed meats and fresh pork products often in combination with O_2 scavengers. However, the lack of fresh beef color development (bright cherry-

red) in vacuum packages is a critical limitation; one which caused researchers to continue the search for an ideal packaging system for fresh beef.

The most recent MAP system for fresh beef is 80% O_2 and 20% CO_2. This is known as a high oxygen system as it contains approximately 4 times the concentration of oxygen as air. This system has the ability to increase shelf life of retail cuts due to a reduction in color deterioration and microbial loads (Borch et. al., 1996). Hunt (2002) reported product packaged in high oxygen systems to have microbial stability of 7 to 12 d while color stability of such products is 7 to 10 d. Increased oxygen concentration is proven to be optimal for blooming and color development of red meat (Bartkowski et. al., 1982; Arensio et. al., 1988; Hunt, 2002;); however, controversy exists on subjects of lipid oxidation and the production of off-odors and flavors. There has been much research supporting accelerated lipid oxidation in high oxygen packaging systems (Taylor, 1985; Jackson et. al., 1992; Jensen et. al., 1998). In contrast, evidence suggesting high oxygen packaging systems have no effect on lipid oxidation rates has been reported (Ordonez and Ledward, 1977; Lopez-Lorenzo et. al., 1980; Arensio et. al., 1988). Aside from the disagreement of lipid oxidation, Shay and Egan (1987) summarized high oxygen packaging research reporting that storage of product in 80 % O_2 and 20 % CO_2 at 5°C resulted in three times the shelf life of controls packaged in conventional overwrap trays. Furthermore, shelf life of conventionally packaged product was limited by discoloration rather than excessive microbial growth. The following table compares new case-ready high oxygen systems with conventional methods.

Table 3: Compare and Contrast of Traditional VS Modified Atmosphere Packaged Retail Packaging Systems (Modified from Hunt, 2002).

	High O_2 MAP	**Vacuum**	**Overwrap**
Color	red	purple	red
Shelf life	7-12 d	weeks	hours to 4-7 d
CO_2 Effect	yes	no	no
Microbial Control	CO_2	aerobic	no
Space	headspace	conserving	conserving

Modified atmosphere packaging systems with the capacity to be utilized in fresh meats are diverse on account of a variety of package materials and gas technology. Examples of applicable systems, other than those previously mentioned include master packs, peelable films particularly useful in red meats, ultra low oxygen (vacuum) with O_2 scavengers, and gas-exchange systems.

Retail Merchandising: Cold Chain Management

Temperature control is a critical factor for maintaining attractive fresh meat color, quality, and microbial soundness; thus, optimizing retail display period and shelf life. Refrigeration can be considered a useful hurdle in retarding microbial growth and product deterioration. Efforts should be made to maintain proper refrigeration temperatures during processing, transportation, storage and display (Segomelo et. al., 2000). The freezing temperature of meat is near $-2^{\circ}C$. Fresh meat deteriorates at a rate directionally proportional to temperature in unfrozen product (Hedrick et. al., 1994). Decreased temperature, approaching but not

below –2°C, suppresses lipid oxidation, color deterioration, enzyme activity, dehydration, and microbial activity.

The key to cold chain management is maintenance of stringent temperature control. A recent audit of U.S. retail refrigeration (Hunt, 2002) showed that the average temperature of retail walk-in storage coolers was 4°C while the average temperature of retail open-topped display cases was 6°C. Disturbingly, Hunt (2002) also reported metmyoglobin accumulation two times faster in fresh beef during retail display at 4.5°C and five times faster at 10°C when compared to control product maintained at 0°C. Additional data revealed shelf life in retail cases could be increased by 12 hours with a significant reduction in spoilage organism growth by decreasing air temperature 1°C.

Retail Merchandising: Microbial Control

Microorganisms directly impact shelf life and more importantly food safety of perishable products. Physical, chemical or microbiological deterioration results in consumer rejection (M^{c}Meekin and Ross, 1996). Foodborne illness results from ingestion of food contaminated by the proliferation of pathogens. Spoilage is measured by exceeding a maximum bacterial level or the development of unacceptable off-odors, off-flavors, or appearance (Borch et. al., 1996). Both spoilage and contamination are controllable with adequate refrigeration temperatures which extend the lag phase of organisms; thereby, minimizing growth and preserving products.

Food safety of fresh meat fundamentally revolves around control of mesophilic microorganisms, having a minimum growth temperature of 10°C (Segomelo et. al., 2000) and psychrotrophic microorganisms, having the ability to grow in temperatures as low as –3°C (Gill and Molin, 1991). It is such organisms that become problematic in temperature abused perishable products. Anaerobic microorganisms are inhibited in the presence of oxygen. Whereas, oxygen is a catalyst for aerobic microorganism growth (Morgan et. al., 1993; Smith et. al., 1996). Vacuum packaging holds a distinct advantage over high oxygen systems in regard to anaerobic bacteria; although the antimicrobial effect of carbon dioxide is substituted in the high oxygen system. It is key to note that less than 10 % of the bacteria initially present on meat can grow at refrigeration temperatures (Mol et. al., 1971; Blickstad et. al., 1981; Jackson et. al., 1992) and fewer anaerobic microorganisms exist with the ability to grow at refrigeration temperatures than do aerobic microorganisms.

The most predominate bacteria associated with spoilage of beef and pork are *Brochothrix thermosphacta, Carnobacterium* spp., *Enterobacteria* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Psudomonas* spp., and *Shewgnella putrefaciens* (Borch et. al., 1996) which result in off-odors, off-flavors, gas production and discoloration. Temperature, gaseous atmosphere, pH, and salt concentration select for certain bacteria and affect growth rate and activity. Incidentally, the composition of muscle and adipose tissue promote bacterial growth of spoilage and pathogenic organisms (Borch et. al., 1996).

Lactoferrin and Case-Ready Beef

Based on currently known functional attributes of LF and the present needs of case-ready beef, the combination of LF and beef has great potential. Bellamy and coworkers (1992a) reported that LF potency was unaffected by the following carbohydrates and proteins at concentrations up to 10 mg/ml: glucose, galactose, fructose, mannose, xylose, maltose, sucrose, lactose, starch, gelatin, and bovine serum albumin. However, LF experienced diminished functionality in the presence of NaCl and KCl at concentrations from 25-100 mmol/L and $MgCl_2$ and $CaCl_2$ at concentrations from 1.0-5.0 mmol/L. The activity of LF in the presence of salt and phosphates will be essential for utilization in enhanced beef products. Lactoferrin has a bicarbonate requirement to uptake iron and form a red pigmented LF-metal complex (Masson and Heremans, 1968). The red hue of the complex has potential to benefit color stability. Carbon dioxide present in air or in high oxygen MAP systems, can fulfill the bicarbonate requirement. Conversely, vacuum package systems combined with impermeable films have only minimal residual CO_2. The amount of residual CO_2 in atmosphere is highly dependent on packaging equipment. It is unknown whether available concentration is sufficient for functionality. In regard to oxidation, Klebanoff and Waltersdorph (1990) reported acceleration in autoxidation of iron as indicated by the disappearance of Fe (II), uptake of O_2, and binding of iron to LF.

Due to LF's activity as a MBA, researchers speculate that LF can form a barrier protecting fresh meat from bacteria present as well as future contamination. The quantity of activated LF required to protect fresh meat is

less than the amount in a single glass of milk (0.1 – 0.3 mg/ml) (Bishop et. al., 1976). Significantly, LF is naturally present in a food product highly consumed by the U.S. population; however, a product responsible for allergies. The most common reaction to milk or milk products is lactose intolerance and is distinctly different from protein intolerances; thus not a threat. The protein content of bovine milk is only 3.4 %. Lactoferrin is a minor protein and constitutes less than 0.6 g/L or less than 2 % of total protein content in milk (Fennema, 1996). Contrastingly, LF has been associated with immune response rather than intolerance.

Upon completion of strict scientific scrutiny, in October of 2001 activated bovine LF was designated by the Food and Drug Administration (FDA) as generally recognized as safe (GRAS) [21 CFR.170.36(f)] at concentrations less than 2 %; equivalent to 65.2 milligrams LF per kg beef (U.S. Food and Drug Administration, 2001). Food ingredients whose use is generally recognized as safe are not required by law to receive FDA approval prior to marketing. Finally, in January of 2002 the USDA approved activated bovine LF for use in fresh beef (Food Safety News, 2002). With regulatory approval complete, activated LF can be applied via high-pressure spray to carcasses, subprimals or retail fresh beef cuts. Ultimately, application can act as a final step in a multiple-hurdle decontamination system to provide safe, wholesome fresh beef from farm to plate.

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CHAPTER III

THE INFLUENCE OF ACTIVATED LACTOFERRIN AS A MICROBIAL BLOCKING AGENT ON SENSORY AND SHELF LIFE CHARACTERISTICS OF CASE-READY FRESH BEEF

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ABSTRACT

Bovine lactoferrin (LF) has been documented to have antibacterial activity against many problematic microorganism associated with fresh beef. Validation of LF as an antimicrobial agent was investigated through application of fresh, case-ready beef strip loin steaks. Organoleptic characteristics were accessed to ensure no adverse affects resulted from LF applications. Mean panelist scores as measured by lean and fat discoloration indicated that a single LF application maintained overall shelf life acceptability longer ($P<0.05$) than non-treated controls. Control Steaks stored 14 d postmortem had approximately 12 h less ($P<0.05$) retail shelf life based on OA scores than the twice LF treated steaks. In contrast, LF/NLF steaks stored 21 d postmortem had approximately 22, 24, and 41 h more ($P<0.05$) retail shelf life than the control, LF/LF, and NLF/LF steaks, respectively. Steaks receiving only subprimal LF application had less lean discoloration ($P<0.05$) than remaining treatment groups and controls.

Similar to OA results, LF/NLF steaks stored 21 d postmortem had approximately 9, 14, and 19 h more ($P<0.05$) retail shelf life based on percentage discoloration scores than the LF/LF, control, and NLF/LF steaks, respectively. Microbial loads, as measured by total plate counts (TPC), increased as postmortem storage time increased. As total plate counts (TPC) increased, overall steak acceptability as rated by trained panelists decreased. The existence of this strong, negative correlation ($r>0.90$) validates the antimicrobial functionality of LF on fresh beef. Sensory results confirmed that organoleptic properties (i.e. tenderness, juiciness, beef flavor, off-flavor) of beef steaks was unaffected by the application and antimicrobial activity of LF. It became evident that as retail display time increased, so did formation of oxidative end products. This was constant regardless of LF treatment in that thiobarbituric acid reactive substances (TBARS) between the various treatments were statistically the same. As a result of improved shelf life and fewer discarded/discounted packages, economic assessment of LF revealed a US $0.036 per kg advantage for treated samples.

INTRODUCTION

Microbial blocking agent (MBA) is a new term applied to a class of naturally occurring defense factors that block microbial adhesion-colonization and growth multiplication (Naidu, 2000ab). Lactoferrin (LF), a heat stable protein first isolated from bovine milk whey (Johansson, 1960; Bullen et. al., 1972) can be classified as a MBA.

The antimicrobial activity of LF was originally attributed to its ability to sequester two atoms of iron (Oram & Reiter, 1961), an essential bacterial nutrient (Chapple et al., 1998), for every one molecule of LF. However, recent discovery of a LF peptide, which is dislocated from the iron binding sites, provides strong evidence of a cidal mechanism that is independent of iron (Dionysius and Milne, 1997).

Due to LF's activity as a MBA, researchers speculate that LF can form a barrier protecting fresh meat from bacteria present as well as future contamination. The quantity of activated LF required to protect fresh meat is less than the amount in a single glass of milk (0.1 – 0.3 mg/ml) (Bishop et. al., 1976). Lactoferrin has been reported to be bacteriostatic against many problematic microorganisms associated with fresh beef, including a broad range of gram-positive bacteria, gram-negative bacteria (Arnold et. al., 1977; Bortner et. al, 1986; Kalmar and Arnold, 1988) including *E. coli* O157:H7 (Jones et. al., 1994), fungi and protozoa (Naidu and Bidlack, 1998).

Lactoferrin requirements bicarbonate to uptake iron, and form a red pigmented LF-metal complex (Masson and Heremans, 1968). The red hue of the complex has potential to benefit color stability. Carbon dioxide present in air or in high oxygen modified atmosphere packaging (MAP) can fulfill the bicarbonate requirement.

The objective of this research was to assess the ability of activated LF to increase retail overall acceptability panel ratings as a result of reduced total plate

counts while retaining desirable organoleptic characteristics of case-ready fresh beef strip loin steaks.

MATERIALS AND METHODS

Sample Collection

USDA Select, A maturity, yield grade 1 and 2 beef carcasses (n = 40) from an unknown origin were selected at random from the Farmland packer/processing facility in Liberal, KS. Paired strip loins were acquired. One of each pair was randomly selected to receive subprimal lactoferrin (LF) treatment. A solution of 2% LF was sprayed (65 mg LF per kg beef) onto subprimals. After treatment, all strip loins (n = 80) were individually identified, vacuum packaged, and transported to Oklahoma State University for further analysis.

Postmortem Handling

Upon arrival to the Food and Agricultural Products Center located on the Oklahoma State University campus, paired strip loin samples were assigned randomly to a postmortem aging treatment of 14, 21, 28, or 35 d. The samples were allowed to age for the respective storage period at refrigeration temperatures (4°C ± 1°C) under vacuum, in the absence of light. At the conclusion of the storage period, each strip loin was fabricated into halves and each half was fabricated into 2.54 cm steaks (n = 4) using sanitized equipment and procedures. One half of each strip loin was chosen randomly to receive

retail LF application (Appendix A). Lactoferrin solution (2% LF) was sprayed onto cut surfaces (5 ml per side) of each assigned steak. Application of retail LF treatment utilized a calibrated (1 ml per trigger release) non-aerosol, plastic spray bottle with an adjustable nozzle. Bottle was calibrated by weighing 1 trigger release of solution.

Sanitation

Sanitation of metal utensils (knives, forceps) was preformed utilizing reagent alcohol and a propane flame between samples. All other equipment (trays, cutting table surfaces) was sanitized utilizing Bi-Quat® (Birko Corporation, Denver, CO) diluted at 200 ppm active quaternary solution, between samples.

Lactoferrin Activation

Isolated bovine lactoferrin was obtained from N-Terminus Laboratories (Pomona, CA). Upon arrival at Oklahoma State University meat science laboratory, the three LF activation powders were stored at room temperature. Twenty-four hours prior to sample treatment, LF was activated. Activation is a multi-step procedure outlined by N-Terminus Laboratories (981 Corporate CTR Dr. # 110 Pomona, CA 91768). Antimicrobial solution contained LF concentration of 2% suspended in a galactose rich polysaccharide.

Retail Shelf Life

Each sample (n = 40 per treatment; treatments = 4) assigned to retail case display was placed in 0.6 ethylene-vinyl alcohol (EVOH) modified atmosphere packaging (MAP) tray (ROCK-TENN Co., Norcross, GA) and sealed with Cryovac 1050 film (Cryovac, Duncan, SC) within 20 minutes of retail fabrication. Packaging utilized a Mondini modified atmosphere packaging machine (Model CV/VG-5, G. Mondini S.P.A. Cologne, Italy). Ten percent of the samples were subjected to an oxygen headspace analyzer (Model HS-750, MOCON Modern Controls Inc., Minneapolis, MN) to ensure that the atmosphere contained 80% O_2. All MAP samples were displayed in commercial retail display coffin cases under cool-white fluorescent light (1,600 to 1,900 lux) at 2 to 4 °C for 14 d. Samples were subjectively evaluated twice daily (0800 and 1700) by a trained panel for lean color (8=bright cherry red; 1=extremely dark brown), fat color (8=creamy white; 1=dark brown or green), percentage discoloration (7=none; 1=complete), and overall appearance (7=extremely desirable, 1=extremely undesirable) (Appendix B).

Total Plate Counts

Total plate counts reflect microbial activity present on and within a sample. Subprimal TPCs (n = 80) were taken from the fat surface of the strip loin after aging, prior to fabrication into steaks. Retail TPCs (n = 320) were taken on the day of fabrication and after 14 d of retail display under MAP. Half of a steak, consisting of lean and fat constituted a sample. All samples were sent overnight

to Food Safety Net Services (San Antonio, TX) for standard total plate counts. Food Safety Net followed standard plating methodology outlined by FDA's Bacteriological Analytical Method (BAM) (Appendix C). Samples were diluted with peptone in a sterile stomacher bag and pummeled for 1 minute. The homogenate was then spiral plated (0.25 mL per plate in quadruplet) onto tryptic soy agar. Plates were incubated at 25°C for 48 hours, counted and reported in TPC per cm^2.

Sensory Analysis

Potential panelists were trained for sensory analysis following American Meat Science Association (1995) guidelines. Trained panelists were subjected to pure activated lactoferrin to identify and establish subsequent flavors associated with the product's lexicon. Steaks (n = 160) were MAP packaged in the same manner as retail shelf life samples for 7 d to allow exposure to modified atmosphere. Samples were then removed from MAP packages and vacuum packed. Steaks were randomly selected for cooking day and order, then tempered for 24 hours at 4°C prior to cooking. Steaks were broiled in an impingement oven (Lincoln Impinger, Model 1132-00-A) at 180 °C to an internal temperature of 70°C (medium degree of doneness). Temperatures were monitored by Digi Sense type T thermocouple (Model 91100-20, Cole-Parmer Instrument Company, Vernon Hills, IL). During product testing, each session consisted of six trained panelists. Sixteen samples were presented to each panelists, allowing a rest break midpoint to reduce fatigue. Two cubic portions

(1.3 cm x 1.3 cm x cooked steak thickness) from each sample were served warm to panelists under red light to. The average of the two portions was recorded. Samples were evaluated for tenderness (8=extremely tender; 1=extremely tough), juiciness (8=extremely juicy; 1=extremely tough), cooked beef flavor (3=strong; 1=not detectable), off flavor (3=strong; 1=not detectable), and overall acceptability (7=extremely desirable; 1=extremely undesirable) (Appendix D). Between samples, panelists cleansed their palate with unsalted cracker and distilled water.

Thiobarbituric Acid Assay

Estimates of lipid oxidation on the surface of samples are made using the thiobarbituric acid (TBA) analysis. Samples (n = 320) were distributed randomly across the three testing d to ensure all treatments were represented. Baseline and final TPCs were taken on d 1 and d 14 of retail display, respectively. The procedure was preformed following protocol outlined by Buege and Aust (1978) (Appendix E, F). The following modifications were made to the procedure: Strip loin samples (10 g) were homogenized with deionized water in a Waring Commercial Blender (Model 33BL79 (700), Waring Products Division Dynamics Corporation of America, New Hartford, Conn.) and centrifuged at 1850 G for 10 minutes at 4°C (Beckman Induction Drive Centerifuge, Model J-6M, Beckman Instruments, Inc., Houston, TX). Two mLs of homogenate, in duplicate, were subjected to TBA reagent and cooked in boiling water bath. After cooling, absorbencies of the supernatant at 531 nm were measured using a

spectrophotometer (Beckman, Model DU 7500). Results were recorded as thiobarbituric acid reactive substances (TBARS) which represent mg malondialdehyde (MDA) equivalents per kg of fresh beef.

Warner-Bratzler Shear Force

Warner-Bratzler shear force value measurements were obtained for each sample as a measurement of tenderness. All samples (n = 160) were placed in individual vacuum packages and frozen until the day of tempering. Steaks were tempered for 24 hours at 4°C prior to cooking. All samples were cooked and tested on a single day to eliminate cooking variation. Steaks were broiled in an impingement oven (Lincoln Impinger, Model 1132-00-A) at 180°C to an internal temperature of 70°C (medium degree of doneness). Temperatures were monitored with a Digi Sense type T thermocouple (Model 91100-20, Cole-Parmer Instrument Company, Vernon Hills, IL). Individual steak weights were obtained prior to and after cooking for the calculation of cooking loss percentages. Upon cooling to 21°C, a minimum of six cores (1.27 cm diameter) were removed parallel to muscle fiber orientation and sheared using the Warner-Bratzler attachment on an Instron Universal Testing Machine (Model 4502, Instron, Canton, MS) at a cross head speed of 200 mm per min. The peak load (kg) of each core was recorded by an IBM PS2 (MODEL 55 SX) utilizing Instron program software. Mean peak load of each sample was calculated and analyzed.

Statistical Analysis

The experiment was a completely randomized design containing a split plot and a 4 X 4 factorial arrangement of treatments. Four levels of postmortem storage time (13, 21, 28, 35 d) and of LF application (control and treatment at subprimal and steak level) existed. Least squares (PROC GLM Version 8, SAS Institute, Cary, NC) was used to measure the effects of postmortem storage time and lactoferrin (LF) treatment on retail shelf life, sensory analysis, thiobarbituric acid analysis, Warner Bratzler shear force, and total plate counts of paired strip loins. A predetermined significance level of $P < 0.05$ was used. Shelf life, TPC, and TBA data were blocked by postmortem storage and retail display d.

RESULTS AND DISCUSSION

Retail Shelf Life

Overall Acceptability. Panelist scores below 4.0 were representative of unacceptable product that would have been discriminated against due to its unfavorable appearance and likely not purchased by consumers (Appendix B). Information included in Table 4 overviews a LF treatment effect on the overall acceptability (OA) of strip loin steaks. Results indicate that a single application of LF (LF/NLF or NLF/LF) was sufficient to statistically improve OA of strip loin steaks when compared to the controls (NLF/NLF) as evaluated by trained panelists. It should be mentioned that the control samples (NLF/NLF) exhibited the lowest numerical ratings in comparison to LF treatments. Table 5 summarizes the number of days steaks remained in the retail display case before

becoming unacceptable as evaluated by a trained panel. Control Steaks stored 14 d postmortem had approximately 12 h less ($P<0.05$) retail shelf life than the twice LF treated steaks. In contrast, LF/NLF steaks stored 21 d postmortem had approximately 22, 24, and 41 h more ($P<0.05$) retail shelf life based on OA scores than the control, LF/LF, and NLF/LF steaks, respectively. Retail display ratings for strip loins steaks stored 14 d postmortem are reported in Table 7. It appeared that spraying LF directly onto subprimals (i.e. LF/NLF and LF/LF) improved retail display time by approximately 2 d when compared to the non-treated control samples. Control samples were rated significantly less desirable ($P<0.05$) by trained panelists as early as retail display d-2 when compared to LF treated samples. Data for strip loins stored postmortem for 21 d appear in Table 8. Similar to the 14 d postmortem storage treatment group, the control steaks (NLF/NLF) received statistically lower OA scores from trained panelists beginning on d-2 of retail display as compared to steaks receiving subprimal LF application (LF/LF and LF/NLF). Dual LF application (LF/LF) gained 1 day of acceptable shelf life when compared to remaining LF treatment groups and control. Control (NLF/NLF), LF/NLF, and NLF/LF samples were unacceptable on d-8 where as LF/LF samples remained acceptable until the 9^{th} day of retail display. In both 14 and 21 d postmortem storage treatment groups control strip loin steaks (NLF/NLF) were consistently less acceptable than those receiving LF application. Information in Table 9 outlines OA scores as rated by a trained panel for strip loins stored postmortem 28 d. Single LF application steaks (LF/NLF and NLF/LF) were unacceptable on d-9 of retail display compared to control

(NLF/NLF) and LF/LF samples which were unacceptable on d-8 of retail display. Control samples (NLF/NLF) that were stored postmortem for 28 d prior to fabrication were statistically less acceptable than LF treated groups immediately after being placed in retail cases on d-1 of display. Table 10 contains OA ratings for strip loin steaks stored 35 d postmortem after observation by a trained panel. Consistent with 28 d postmortem storage findings, 35 d stored steaks receiving a single LF application (LF/NLF and NLF/LF) displayed improved OA when compared to control (NLF/NLF) and twice LF treated samples (LF/LF).

Percent Discoloration. A LF treatment effect was present in percent lean discoloration mean panelist scores, in that samples receiving only subprimal LF application (LF/NLF) had statistically less lean surface discoloration than the remaining treatment groups (Table 4). Additionally, summarized data imply that retail steak LF application (LF/LF and NLF/LF) results in greater ($P<0.05$) discoloration when compared to remaining LF treated samples (LF/NLF). Table 6 overviews the number of days steaks remained in the retail display case before becoming 1-10% discolored as evaluated by a trained panel. Similar to OA results, LF/NLF steaks stored 21 d postmortem had approximately 9, 14, and 19 h more ($P<0.05$) retail shelf life based on percentage discoloration scores than the LF/LF, control, and NLF/LF steaks, respectively. Data presented in Tables 11 through 14 revealed few differences early in retail display. However, results notably indicate that single LF application (LF/NLF and NLF/LF) retarded lean discoloration during critical retail display d when samples were approaching unacceptability thresholds associated with longer retail display times.

Discoloration of perishable fresh meat products is the primary basis of purchase intent. Consumers associate beef that is not bright cherry-red as unacceptable from a wholesomeness and freshness standpoint. When the percentage of oxidized myoglobin reaches 40 to 60 % muscle tissue will begin to exhibit characteristics of pigment discoloration and appear brown (Lawrie, 1985). It is then products are discounted in price, discarded, or reworked into further processed items. The ability to increase color stability and reduce discoloration from spoilage holds great profit potential.

Lean Color. Although LF treatment groups were statistically similar, as postmortem storage time increased, observed lean color on d-1 of retail display became less desirable. Tables 15 through 18 summarize lean color scores of strip loin steaks as rated by trained panelist. It should be pointed out that single LF treated samples (LF/ NLF and NLF/LF) exhibited more desirable lean color when compared to twice LF treated and control steaks in postmortem storage treatments 14, 21, and 28. Control steaks (NLF/NLF) from strip loins stored 35 d postmortem were consistently rated the lowest when compared to LF treated samples.

Fat Color. No differences ($P<0.05$) exist for mean panelist fat color scores as evaluated by trained panelists among LF treatment within a single retail display day. However, similar to lean color data, d-1 retail display fat color ratings decreased as postmortem storage time increased, particularly following 21 or 28 d postmortem storage (Tables 19 to 22).

Total Plate Counts

To eliminate a three-way interaction and confoundness, data were analyzed independent of postmortem storage time (14, 21, 28, or 35 d) for LF treatment effects. Baseline total plate counts (TPC) were obtained on d 1 of retail display and used for comparison. Information contained in Table 23 implies that microbial loads increased with increased postmortem storage regardless of LF treatment. It is speculated to be a direct result of longer exponential and stationary growth phases. After 14 d of retail display, control samples (NLF/NLF) despite postmortem storage treatment, exhibited statistically higher TPC than LF treated samples.

Table 24 displays the effect of LF application on microbial growth of strip loin samples stored 14 d postmortem. Control samples (NLF/NLF) exhibited statistically higher TPC after 14 d of retail display when compared to samples receiving retail LF application (LF/LF and NLF/LF). Interestingly, as presented in Tables 25 and 26, TPC for samples from the 21 and 28 d postmortem storage periods, the LF/NLF exhibited the lowest TPC upon completion of retail display. Lactoferrin treated steaks stored for 35 d postmortem resulted in significantly lower ($P<0.05$) baseline TPC than the control (NLF/NLF) (Table 27). It should be mentioned that control samples (NLF/NLF) consistently had numerically higher initial TPC than did LF treated samples.

Microbial spoilage of perishable products is inevitable. Extending shelf life has great potential to increase profits. Pathogenic contamination is also a viable

threat, which can be costly in the event of an outbreak and a recall. Controlling microbiological soundness reduces such risks and gains optimal shelf life.

Shelf Life and Total Plate Count Correlation

The antimicrobial activity of LF has been documented against many problematic bacteria associated with fresh beef. However, minimal research has been conducted applying LF to fresh meat surfaces. To determine the association between total plate counts (spoilage microorganism) and retail shelf life overall acceptability, correlation coefficients were determined (Table 28). Shelf life and TPC data had a strong ($r > .90$), negative relationship, particularly d-1 through d-9 of retail display, with decreasing correlation as retail display day increased (Table 28). That is, as TPC increased, overall acceptability as rated by trained panelists decreased. Control samples (NLF/NLF) had the highest TPC and the lowest appearance scores. Furthermore, shelf life and d-14 retail TPC data established greater association than retail d-1 TPC. The existence of this correlation validates the antimicrobial functionality of LF on fresh beef.

A survey conducted by CIES (2002), an international forum for major food industry professionals, reported that retailers number one concern was food safety and security. Case-ready combined with effective antimicrobials provide a solution. Eliminating fabrication at the retail level, case-ready merchandisers decrease the risk of contamination thereby removing responsibility from the retail store chain. As a result, retailers are shifting to case-ready, selling 1.2 billion

A postmortem storage by LF treatment interaction was present ($P<0.05$) in juiciness and off-flavor ratings as scored by a trained panel; however, no consistent results were evident to suspect adverse effects of lactoferrin on cooked beef juiciness or off-flavor presented in Tables 31 and 32, respectively. All LF treatment and postmortem storage groups were statistically similar ($P>0.05$) in regard to mean panelist flavor scores (Table 33). These sensory data and results confirm that preferred organoleptic properties of beef steaks remain unaffected by the application and antimicrobial activity of LF.

Lipid Oxidation

The spontaneous reaction of atmospheric oxygen and organic compounds yields degradative changes affecting the shelf life of a fresh meat product. One such reaction is that of lipid oxidation which occurs in stored and displayed meat products. Reducing lipid oxidation is a driving force behind extending fresh product retail shelf life. One indicator of the presence of lipid oxidation is the presence of thiobarbituric acid reactive substances (TBARS). Many research investigations have characterized meat samples having a TBARS level of 1.0 as having oxidative flavors that could be detected by trained consumer panelists.

Most modified atmosphere packaging systems utilized purified oxygen that promotes oxidation of fresh meat samples. Many commercially available case-ready fresh beef systems utilize various antioxidants, which will retard the formation of oxidation end products. In this investigation it became very evident that as retail display time increased so did formation of oxidative end products.

This was constant regardless of LF treatment in that no significant differences ($P>0.05$) were observed between the various treatments (Table 34). Following 14 d of retail display, all strip loin samples displayed TBARS concentration levels well above the reported sensory panelist detection level (i.e., 1.0). However, it is important to note that none of the treatment means were categorized as being unacceptable from an off-flavor standpoint in sensory panel after 7 d of retail display.

Objective Tenderness

Postmortem aging results in enzymatic degradation of muscle fibers; thus, increased tenderness. A postmortem storage by LF treatment interaction was observed ($P<0.05$). Warner-Bratzler shear force means are stratified by postmortem storage (14, 21, 28, 35 d) between LF treatments in Table 35 to emphasize that no LF treatment effect existed. Samples receiving only subprimal LF displayed the greatest response to postmortem aging when compared to control steaks. This implies LF does not adversely affect tenderness fresh beef processed under current industry postmortem handling and storage methods.

Economic Assessment of Lactoferrin

One of the many challenges in developing technology is the ability to make it economically feasible. In an attempt to estimate the economical impact of LF on enhancing retail display life through improving the microbiological

soundness of case-ready beef cuts, percentages were calculated of beef cuts which were categorized as being undesirable due to surface discoloration, fat color or general overall appearance (Table 36). These undesirable products would have been discounted or even discarded as a result of their inferior appearance.

The second phase in attempting to assess the economical impact of LF on beef in a case-ready system was to estimate the value of closely-trimmed cuts and lean trimmings from a typical beef carcass. Using the average beef carcass produced in the U.S. (YG 2.9, Select quality grade, 370 kg, 63.75% dressing percentage) as determined from the latest National Beef Quality Audit (National Beef Cattleman's Association, 2000), an economic value was calculated. The average boxed beef prices for 2000 were utilized in the Oklahoma State University (OSU) Boxed Beef Yield Value Calculator (Dolezal et. al., 1995) and gross carcass values were determined (Appendix E, F, G).

According to the OSU Boxed Beef Calculator, estimates for the base carcass, with no discarded packages was $995.30 (Table 37). This represents approximately 243 kg (65.91% box beef yield) of the original 370 kg carcass. As display day increased, a greater percentage of case-ready packages were being pulled and discarded from the operation. For example, on day 6 of retail display, 79.3% ($789.27) of the original LF treated retail packages were still available for sale whereas only 75% ($746.48) of the control (NLF/NLF) packages were still available for purchase at their full retail value. This difference represents a $42.80 per carcass equivalent advantage for the LF treated carcass. If this

soundness of case-ready beef cuts, percentages were calculated of beef cuts which were categorized as being undesirable due to surface discoloration, fat color or general overall appearance (Table 36). These undesirable products would have been discounted or even discarded as a result of their inferior appearance.

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According to the OSU Boxed Beef Calculator, estimates for the base carcass, with no discarded packages was $995.30 (Table 37). This represents approximately 243 kg (65.91% box beef yield) of the original 370 kg carcass. As display day increased, a greater percentage of case-ready packages were being pulled and discarded from the operation. For example, on day 6 of retail display, 79.3% ($789.27) of the original LF treated retail packages were still available for sale whereas only 75% ($746.48) of the control (NLF/NLF) packages were still available for purchase at their full retail value. This difference represents a $42.80 per carcass equivalent advantage for the LF treated carcass. If this

advantage were prorated over the 243 kg of carcass yield from the average carcass, this would represent approximately US $0.036 per kg economical advantage for the LF treated samples.

IMPLICATIONS

Activated LF can be applied via high-pressure spray to carcasses, subprimals or retail fresh beef cuts. Ultimately, application can act as a final step in a multiple-hurdle decontamination system to provide safe, wholesome fresh beef from farm to plate. Importantly, no detrimental sensory effects are encountered when LF is incorporated into a case-ready beef system. Shelf life stability of LF treated steaks was improved when compared to conventional case-ready beef systems. In October of 2001 activated LF was designated by the Food and Drug Administration (FDA) as generally recognized as safe (GRAS) [21 CFR.170.36(f)] at concentrations equal to or less than 2 %; equivalent to 65.2 milligrams LF per kg beef (U.S. Food and Drug Administration, 2001). Food ingredients whose use is GRAS are not required by law to receive FDA approval prior to marketing. Finally, in January of 2002 the United States Department of Agriculture (USDA) approved activated bovine LF as an ingredient for use in fresh beef.

Table 4: Effects of Lactoferrin (LF) Application on Overall Acceptability[1] and Percentage Discoloration[2] of Case-Ready Strip Loin Steaks

	Treatment[3]			
	LF / LF	LF / NLF	NLF / LF	NLF / NLF
Overall Acceptability	5.14[ab]	5.33[a]	5.35[a]	4.97[b]
Percent Discoloration	4.39[b]	4.52[a]	4.38[b]	4.41[b]

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] Overall Acceptability: 7= Extremely Desirable, 6= Desirable, 5= Slightly, Desirable, 4= Acceptable, 3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable

[2] Percent Discoloration: 7= None, 6= 1-10%, 5= 11-25%, 4= 26-50%, 3= 51-75%, 2= 76-99%, 1= Complete.

[3] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 5: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach Unacceptable

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	7.05±0.15^{a}	6.87±0.16^{ab}	6.85±0.16^{ab}	6.62±0.16^{b}
21	8.34±0.20^{b}	9.35±0.23^{a}	7.65±0.25^{c}	8.41±0.23^{b}
28	7.75±0.22	7.75±0.21	7.64±0.28	7.76±0.27
35	6.77±0.22	6.43±0.26	6.58±0.29	6.19±0.25

^{abc} Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 6: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach 1-10% Discoloration

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	5.63±0.13	5.57±0.12	5.56±0.13	5.58±0.12
21	5.58±0.13^{b}	5.97±0.14^{c}	5.16±0.13^{a}	5.38±0.13^{b}
28	5.79±0.18	5.73±0.15	5.89±0.15	5.61±0.16
35	5.49±0.19	5.32±0.18	5.23±0.20	5.06±0.19

^{abc} Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 5: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach Unacceptable

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	7.05±0.15[a]	6.87±0.16[ab]	6.85±0.16[ab]	6.62±0.16[b]
21	8.34±0.20[b]	9.35±0.23[a]	7.65±0.25[c]	8.41±0.23[b]
28	7.75±0.22	7.75±0.21	7.64±0.28	7.76±0.27
35	6.77±0.22	6.43±0.26	6.58±0.29	6.19±0.25

[abc] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 6: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach 1-10% Discoloration

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	5.63±0.13	5.57±0.12	5.56±0.13	5.58±0.12
21	5.58±0.13[b]	5.97±0.14[c]	5.16±0.13[a]	5.38±0.13[b]
28	5.79±0.18	5.73±0.15	5.89±0.15	5.61±0.16
35	5.49±0.19	5.32±0.18	5.23±0.20	5.06±0.19

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[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

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28	7.75±0.22	7.75±0.21	7.64±0.28	7.76±0.27
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Table 6: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach 1-10% Discoloration

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	5.63±0.13	5.57±0.12	5.56±0.13	5.58±0.12
21	5.58±0.13[b]	5.97±0.14[c]	5.16±0.13[a]	5.38±0.13[b]
28	5.79±0.18	5.73±0.15	5.89±0.15	5.61±0.16
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[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

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	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	7.05±0.15[a]	6.87±0.16[ab]	6.85±0.16[ab]	6.62±0.16[b]
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28	7.75±0.22	7.75±0.21	7.64±0.28	7.76±0.27
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[abc] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 6: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach 1-10% Discoloration

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	5.63±0.13	5.57±0.12	5.56±0.13	5.58±0.12
21	5.58±0.13[b]	5.97±0.14[c]	5.16±0.13[a]	5.38±0.13[b]
28	5.79±0.18	5.73±0.15	5.89±0.15	5.61±0.16
35	5.49±0.19	5.32±0.18	5.23±0.20	5.06±0.19

[abc] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 5: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach Unacceptable

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	7.05±0.15[a]	6.87±0.16[ab]	6.85±0.16[ab]	6.62±0.16[b]
21	8.34±0.20[b]	9.35±0.23[a]	7.65±0.25[c]	8.41±0.23[b]
28	7.75±0.22	7.75±0.21	7.64±0.28	7.76±0.27
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[abc] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 6: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach 1-10% Discoloration

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	5.63±0.13	5.57±0.12	5.56±0.13	5.58±0.12
21	5.58±0.13[b]	5.97±0.14[c]	5.16±0.13[a]	5.38±0.13[b]
28	5.79±0.18	5.73±0.15	5.89±0.15	5.61±0.16
35	5.49±0.19	5.32±0.18	5.23±0.20	5.06±0.19

[abc] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 5: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach Unacceptable

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	7.05±0.15[a]	6.87±0.16[ab]	6.85±0.16[ab]	6.62±0.16[b]
21	8.34±0.20[b]	9.35±0.23[a]	7.65±0.25[c]	8.41±0.23[b]
28	7.75±0.22	7.75±0.21	7.64±0.28	7.76±0.27
35	6.77±0.22	6.43±0.26	6.58±0.29	6.19±0.25

[abc] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 6: Effect of LF Treatment on Retail Shelf Life (d) of Strip Loin Steaks within Postmortem Storage Time: LS Means for Days to Reach 1-10% Discoloration

	Treatment[1]			
Postmortem Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	5.63±0.13	5.57±0.12	5.56±0.13	5.58±0.12
21	5.58±0.13[b]	5.97±0.14[c]	5.16±0.13[a]	5.38±0.13[b]
28	5.79±0.18	5.73±0.15	5.89±0.15	5.61±0.16
35	5.49±0.19	5.32±0.18	5.23±0.20	5.06±0.19

[abc] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 7: Overall Acceptability Scores[1] for Retail Display Steaks Stored 14 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	7.00	7.00
2	6.90[a]	6.86[a]	6.87[a]	6.77[b]
3	6.58[a]	6.37[ab]	6.22[b]	6.18[b]
4	5.38[a]	5.37[a]	5.58[a]	4.63[b]
5	4.88[a]	4.87[a]	4.43[b]	4.35[b]
6	4.81[a]	4.94[a]	4.81[a]	3.87[b]
7	4.32[a]	4.39[a]	3.70[b]	3.51[b]
8	3.26	3.11	3.32	2.93
9	2.82	2.58	2.55	2.63
10	2.46[a]	2.04[b]	2.04[b]	2.11[b]
11	1.59	1.57	1.64	1.70
12	1.22	1.22	1.22	1.22
13	1.35	1.33	1.41	1.46
14	1.27	1.35	1.33	1.43

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Overall Acceptability: 7= Extremely Desirable, 6= Desirable, 5= Slightly, Desirable, 4= Acceptable, 3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 8: Overall Acceptability Scores[1] for Retail Display Steaks Stored 21 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	6.99	6.98
2	6.99^{a}	6.97^{ab}	6.94^{bc}	6.91^{c}
3	6.62^{c}	6.57^{ab}	6.46^{bc}	6.36^{a}
4	6.01^{ab}	6.11^{a}	5.90^{b}	5.82^{b}
5	5.75	5.69	5.67	5.61
6	5.88	5.90	5.83	5.87
7	4.84^{ab}	5.08^{a}	4.73^{bc}	4.32^{c}
8	4.17	3.93	3.42	3.61
9	2.89^{a}	2.83^{a}	1.88^{b}	2.08^{b}
10	2.13^{a}	2.10^{ab}	1.43^{c}	1.74^{bc}
11	1.55^{b}	1.91^{a}	1.20^{c}	1.58^{b}
12	1.30	1.55	1.13	1.39
13	1.33^{bc}	1.86^{a}	1.05^{c}	1.47^{ab}
14	1.32^{a}	1.51^{a}	1.00^{b}	1.29^{b}

^{abc} Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Overall Acceptability: 7= Extremely Desirable, 6= Desirable, 5= Slightly, Desirable, 4= Acceptable, 3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 8: Overall Acceptability Scores[1] for Retail Display Steaks Stored 21 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	6.99	6.98
2	6.99^{a}	6.97^{ab}	6.94^{bc}	6.91^{c}
3	6.62^{c}	6.57^{ab}	6.46^{bc}	6.36^{a}
4	6.01^{ab}	6.11^{a}	5.90^{b}	5.82^{b}
5	5.75	5.69	5.67	5.61
6	5.88	5.90	5.83	5.87
7	4.84^{ab}	5.08^{a}	4.73^{bc}	4.32^{c}
8	4.17	3.93	3.42	3.61
9	2.89^{a}	2.83^{a}	1.88^{b}	2.08^{b}
10	2.13^{a}	2.10^{ab}	1.43^{c}	1.74^{bc}
11	1.55^{b}	1.91^{a}	1.20^{c}	1.58^{b}
12	1.30	1.55	1.13	1.39
13	1.33^{bc}	1.86^{a}	1.05^{c}	1.47^{ab}
14	1.32^{a}	1.51^{a}	1.00^{b}	1.29^{b}

^{abc} Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Overall Acceptability: 7= Extremely Desirable, 6= Desirable, 5= Slightly, Desirable, 4= Acceptable, 3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 9: Overall Acceptability Scores[1] for Retail Display Steaks Stored 28 Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00[a]	7.00[a]	7.00[a]	6.89[b]
2	7.00[a]	6.99[a]	7.00[a]	6.96[b]
3	6.65	6.56	6.52	6.46
4	6.09	6.24	6.34	6.23
5	5.42	5.52	5.62	5.46
6	5.03	5.18	5.56	5.03
7	4.58[c]	5.28[ab]	5.39[a]	4.78[bc]
8	3.79	4.08	4.26	3.74
9	2.93	3.10	2.89	2.73
10	2.10	2.43	2.24	2.13
11	1.83	2.04	1.76	1.60
12	1.56	1.43	1.41	1.39
13	1.88	1.75	1.61	1.56
14	1.67	1.47	1.33	1.41

[abc] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Overall Acceptability: 7= Extremely Desirable, 6= Desirable, 5= Slightly, Desirable, 4= Acceptable, 3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 10: Overall Acceptability Scores[1] for Retail Display Steaks Stored 35 Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	7.00	6.97
2	7.00	7.00	6.96	6.98
3	6.50	6.44	6.43	6.35
4	5.33	5.50	5.72	5.43
5	5.24	5.02	5.04	7.78
6	4.72^{a}	4.00^{b}	4.22^{ab}	3.72^{b}
7	3.70^{a}	4.00^{a}	4.00^{a}	2.96^{b}
8	2.98^{a}	2.48^{b}	2.25^{bc}	1.97^{c}
9	1.83	1.67	1.58	1.42
10	1.14	1.24	1.11	1.19
11	1.02	1.08	1.03	1.13
12	1.00^{b}	1.00^{b}	1.00^{b}	1.09^{a}
13	1.00^{b}	1.00^{b}	1.00^{b}	1.11^{a}
14	1.00^{b}	1.00^{b}	1.00^{b}	1.11^{a}

^{abc} Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Overall Acceptability: 7= Extremely Desirable, 6= Desirable, 5= Slightly, Desirable, 4= Acceptable, 3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 11: Percent Discoloration Scores[1] for Retail Display Steaks Stored 14 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	7.00	7.00
2	6.95	6.97	6.93	6.97
3	6.63	6.57	6.52	6.52
4	6.38	6.35	6.30	6.20
5	5.94[ab]	6.18[a]	5.81[b]	5.78[b]
6	5.76	5.77	5.50	5.70
7	5.19	5.47	5.33	5.27
8	3.80	4.15	4.05	4.02
9	3.37	3.52	3.47	3.59
10	2.68	2.89	2.88	2.41
11	2.20	2.30	2.38	2.41
12	1.89	1.89	1.96	2.17
13	1.70	1.67	1.80	1.93
14	1.65	1.70	1.68	1.86

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Percent Discoloration: 7= None, 6= 1-10%, 5= 11-25%, 4= 26-50%, 3= 51-75%, 2= 76-99%, 1= Complete.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 12: Percent Discoloration Scores[1] for Retail Display Steaks Stored 21 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	7.00	7.00
2	7.00	7.00	6.99	6.98
3	6.81	6.78	6.77	6.75
4	6.63	6.63	6.62	6.58
5	6.77	6.81	6.72	6.78
6	6.15	6.26	6.15	6.24
7	5.01	5.29	4.87	4.95
8	4.20	4.45	3.97	4.11
9	2.84	3.13	2.62	2.81
10	2.24^{bc}	2.70^{a}	1.89^{c}	2.21^{b}
11	1.89^{ab}	2.43^{c}	1.58^{a}	1.97^{b}
12	1.58^{b}	1.98^{a}	1.37^{b}	1.69^{ab}
13	1.65^{b}	2.14^{a}	1.23^{b}	1.67^{ab}
14	1.40^{a}	1.66^{a}	1.04^{b}	1.36^{a}

[abc] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Percent Discoloration: 7= None, 6= 1-10%, 5= 11-25%, 4= 26-50%, 3= 51-75%, 2= 76-99%, 1= Complete.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 13: Percent Discoloration Scores[1] for Retail Display Steaks Stored 28 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	7.00	6.96
2	7.00	6.99	7.00	6.96
3	7.00	6.98	6.98	6.95
4	6.46	6.60	6.69	6.57
5	6.01	6.11	6.20	6.16
6	5.41	5.70	6.11	5.69
7	4.35[b]	5.15[a]	5.41[ab]	4.80[ab]
8	3.92	4.52	4.74	4.35
9	2.85	3.66	3.51	3.56
10	2.46	2.95	2.85	2.67
11	2.03	2.24	2.02	1.84
12	1.92	1.85	1.67	1.69
13	2.06	2.05	2.17	1.83
14	1.96	1.87	1.85	1.63

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Percent Discoloration: 7= None, 6= 1-10%, 5= 11-25%, 4= 26-50%, 3= 51-75%, 2= 76-99%, 1= Complete.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 14: Percent Discoloration Scores[1] for Retail Display Steaks Stored 35 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.00	7.00	6.97	7.00
2	7.00	7.00	7.00	6.96
3	7.00	7.00	7.00	7.00
4	5.96^{c}	6.56^{ab}	6.63^{a}	6.19^{bc}
5	5.93	5.80	5.80	5.65
6	5.06	5.17	5.22	5.06
7	4.56	4.41	4.19	3.85
8	2.87	3.10	2.81	2.51
9	2.00	2.14	1.86	1.81
10	1.63	1.68	1.52	1.63
11	1.41	1.48	1.25	1.37
12	1.07	1.16	1.07	1.13
13	1.04	1.07	1.04	1.15
14	1.00^{b}	1.00^{b}	1.00^{b}	1.11^{a}

^{ab} Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Percent Discoloration: 7= None, 6= 1-10%, 5= 11-25%, 4= 26-50%, 3= 51-75%, 2= 76-99%, 1= Complete.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 15: Lean Color Scores[1] for Retail Display Steaks Stored 14 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.98	8.00	7.98	8.00
2	7.53	7.49	7.33	7.34
3	6.98	6.90	6.91	6.87
4	5.75^{ab}	5.58^{bc}	5.86^{a}	5.43^{c}
5	5.44^{a}	5.55^{a}	5.40^{b}	5.16^{b}
6	5.31	5.43	5.21	5.11
7	4.92	5.04	4.79	4.74
8	3.70	4.02	3.74	3.76
9	3.20	3.32	3.28	3.33
10	2.53	2.67	2.65	2.84
11	1.99	2.04	2.19	2.29
12	1.61	1.63	1.74	1.83
13	1.61	1.61	1.80	1.89
14	1.63	1.62	1.73	1.92

[abc] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Lean Color: 8= Bright Cherry-Red, 7= Moderately Bright Cherry, 6= Cherry-Red, 5= Slightly Dark Red, 4= Moderately Dark Red or Brown, 3= Dark Red or Brown, 2= Very Dark Brown, 1= Extremely Dark Brown.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 16: Lean Color Scores[1] for Retail Display Steaks Stored 21 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	8.00[a]	8.00[a]	7.97[a]	7.81[b]
2	7.90[a]	7.79[a]	7.80[a]	7.64[b]
3	7.05	6.88	6.85	6.81
4	6.53	6.30	6.33	6.16
5	5.78	5.57	5.62	5.46
6	6.05	5.98	5.77	5.72
7	5.30	5.39	5.07	5.00
8	4.36	4.75	4.15	4.26
9	3.03[ab]	3.29[a]	2.75[b]	2.81[b]
10	2.34[b]	2.81[a]	1.94[c]	2.21[cb]
11	1.94[b]	2.46[a]	1.58[c]	2.00[b]
12	1.63[ab]	2.05[a]	1.28[b]	1.72[a]
13	2.01	2.32	1.60	2.58
14	1.48[a]	1.71[a]	1.06[b]	1.38[ab]

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Lean Color: 8= Bright Cherry-Red, 7= Moderately Bright Cherry, 6= Cherry-Red, 5= Slightly Dark Red, 4= Moderately Dark Red or Brown, 3= Dark Red or Brown, 2= Very Dark Brown, 1= Extremely Dark Brown.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 17: Lean Color Scores[1] for Retail Display Steaks Stored 28 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.91[a]	7.37[b]	7.78[a]	7.44[b]
2	7.74[a]	7.54[bc]	7.63[b]	7.41[c]
3	6.69	6.77	6.77	6.69
4	6.18[ab]	6.03[b]	6.38[a]	5.98[b]
5	5.56[b]	5.59[b]	5.95[a]	5.58[b]
6	5.53	5.65	5.97	5.53
7	5.04	5.35	5.56	5.28
8	3.71	4.12	4.31	3.89
9	2.73	3.16	3.20	2.80
10	2.50	2.92	2.73	2.70
11	2.03	2.30	2.16	1.82
12	1.75	1.77	1.83	1.69
13	2.06	1.95	2.22	1.83
14	2.25	2.13	2.19	2.07

[abc] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Lean Color: 8= Bright Cherry-Red, 7= Moderately Bright Cherry, 6= Cherry-Red, 5= Slightly Dark Red, 4= Moderately Dark Red or Brown, 3= Dark Red or Brown, 2= Very Dark Brown, 1= Extremely Dark Brown.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 18: Lean Color Scores[1] for Retail Display Steaks Stored 35 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.75	7.67	7.75	7.61
2	7.22	7.09	6.87	6.98
3	5.83	5.65	5.67	5.60
4	5.13	5.33	5.48	5.15
5	5.35	5.33	5.36	5.18
6	4.89	4.83	4.56	4.44
7	4.04	4.07	3.96	3.74
8	3.00	3.13	2.90	2.68
9	1.97	2.17	1.97	1.81
10	1.59	1.71	1.48	1.59
11	1.43	1.56	1.29	1.44
12	1.04	1.11	1.04	1.11
13	1.00[b]	1.00[b]	1.00[b]	1.11[a]
14	1.00	1.00	1.00	1.04

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Lean Color: 8= Bright Cherry-Red, 7= Moderately Bright Cherry, 6= Cherry-Red, 5= Slightly Dark Red, 4= Moderately Dark Red or Brown, 3= Dark Red or Brown, 2= Very Dark Brown, 1= Extremely Dark Brown.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 19: Fat Color Scores[1] for Retail Display Steaks Stored 14 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	8.00	8.00	7.97	8.00
2	7.28	7.16	7.25	7.18
3	6.50	6.15	6.38	6.23
4	5.33[a]	4.96[b]	5.20[a]	5.01[b]
5	4.58	4.49	4.37	4.31
6	4.35	4.37	4.24	4.19
7	3.89	3.86	3.82	3.76
8	3.07	3.12	3.11	3.12
9	3.07	3.09	3.03	3.11
10	2.83	2.93	2.86	2.93
11	2.20	2.13	2.16	2.22
12	1.41	1.43	1.50	1.56
13	1.20	1.22	1.33	1.37
14	1.71	1.70	1.67	1.75

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Fat Color: 8= Creamy White, 7= Mostly Creamy White, 6= Slightly Tan, 5= Tan, 4= Slightly Brown, 3= Moderately Brown, 2= Brown or Slightly Green, 1= Dark Brown or Green.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 20: Fat Color Scores[1] for Retail Display Steaks Stored 21 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.44	7.43	7.43	7.41
2	7.31	7.31	7.30	7.31
3	6.60	6.48	6.60	6.43
4	5.71	5.67	5.65	5.56
5	4.82	4.95	4.87	4.67
6	5.10	5.07	5.12	4.96
7	4.87	4.90	4.67	4.65
8	4.37[a]	4.31[a]	4.10[ab]	3.96[b]
9	3.12	2.99	2.95	2.85
10	2.77	2.68	2.63	2.56
11	2.49	2.54	2.32	2.42
12	2.10	2.07	2.00	2.00
13	2.00	2.00	2.00	2.00
14	1.76	1.77	1.54	1.69

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] Fat Color: 8= Creamy White, 7= Mostly Creamy White, 6= Slightly Tan, 5= Tan, 4= Slightly Brown, 3= Moderately Brown, 2= Brown or Slightly Green, 1= Dark Brown or Green.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 21: Fat Color Scores[1] for Retail Display Steaks Stored 28 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.33	7.33	7.41	7.33
2	7.41	7.45	7.39	7.44
3	5.94	6.02	6.09	6.09
4	5.40	5.59	5.71	5.60
5	4.68	4.77	4.95	4.79
6	4.72[b]	4.80[b]	4.97[a]	4.78[b]
7	4.75	4.77	5.06	4.89
8	3.81	4.05	4.24	3.96
9	2.83	3.06	3.16	3.07
10	2.54	2.88	2.80	2.72
11	2.65	2.80	2.80	2.80
12	1.77	1.85	1.76	1.78
13	2.00	2.00	2.00	2.00
14	2.21	2.56	2.59	2.52

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Fat Color: 8= Creamy White, 7= Mostly Creamy White, 6= Slightly Tan, 5= Tan, 4= Slightly Brown, 3= Moderately Brown, 2= Brown or Slightly Green, 1= Dark Brown or Green.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 22: Fat Color Scores[1] for Retail Display Steaks Stored 35 d Prior to Fabrication as Influenced by Lactoferrin Application

	Treatment[2]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	7.50	7.50	7.50	7.50
2	6.83	6.83	6.83	6.83
3	6.63	6.54	6.67	6.48
4	4.89^{c}	5.31^{ab}	5.50^{a}	5.02^{bc}
5	5.02	5.09	5.00	4.91
6	4.94	4.89	5.00	5.00
7	4.07	4.07	4.11	4.04
8	3.87	3.90	3.86	3.83
9	3.14	3.22	3.14	3.14
10	3.06	2.95	3.00	2.94
11	2.43	2.43	2.43	2.35
12	2.00	2.00	2.00	2.00
13	1.33	1.33	1.33	1.33
14	1.33	1.33	1.33	1.33

^{abc} Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Fat Color: 8= Creamy White, 7= Mostly Creamy White, 6= Slightly Tan, 5= Tan, 4= Slightly Brown, 3= Moderately Brown, 2= Brown or Slightly Green, 1= Dark Brown or Green.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 23: The effects of Lactoferrin (LF) Application on Total Plate Counts (TPC/g) of Case-Ready Strip Loin Samples Stratified by Postmortem Storage and Retail Display Times

	Treatment[1]			
Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	33,763[a]	89,449[ab]	59,458[a]	147,359[b]
21	103,905	107,399	285,186	368,782
28	81,924[ab]	43,204[a]	122,300[b]	100,031[ab]
35	2,029,590[a]	2,026,925[a]	2,042,650[ab]	2,073,100[b]
Retail Display, d				
1	19,787	39,387	35,177	86,132
14	145,227[a]	393,249[a]	138,553[a]	>4,000,000[b]

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 24: The Effects of Lactoferrin (LF) Application on Total Plate Counts (TPC/g) of Case-Ready Strip Loin Samples Stored 14 d

	Treatment[1]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	6,192	6,087	12,029	54,841
14	61,333[a]	172,812[ab]	106,887[a]	239,877[b]

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 25: The Effects of Lactoferrin (LF) Application on Total Plate Counts (TPC/g) of Case-Ready Strip Loin Samples Stored 21 d

	Treatment[1]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	1,701	97,021	14,595	44,230
14	206,109	117,777	555,777	693,333

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 26: The Effects of Lactoferrin (LF) Application on Total Plate Counts (TPC/g) of Case-Ready Strip Loin Samples Stored 28 d

	Treatment[1]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	31,892	17,709	72,377	18,730
14	131,975[ab]	68,700[a]	172,222[b]	181,333[b]

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 27: The Effects of Lactoferrin (LF) Application on Total Plate Counts (TPC/g) of Case-Ready Strip Loin Samples Stored 35 d

	Treatment[1]			
Retail Display, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
1	59,180[a]	53,850[a]	85,300[a]	146,200[b]
14	>4,000,000	>4,000,000	>4,000,000	>4,000,000

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 28: Correlation (r) Between Total Plate Counts (TPC/g) and Overall Retail Appearance Scores for Case-Ready Strip Loin Steaks

	Overall Appearance, d						
TPC, d	1	3	5	7	9	11	13
1	-.87	-.97	-.92	-.61	-.73	-.15	-.33
14	-.91	-.88	-.93	-.81	-.92	-.51	-.29

Table 29: The Influence of Lactoferrin (LF) Application on the Sensory Panelist Overall Acceptability Scores[1] of Strip Loin Steaks

Treatment[2]			
LF / LF	LF / NLF	NLF / LF	NLF / NLF
5.14[ab]	5.33[a]	5.35[a]	4.97[b]

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] Overall Acceptability: 7= Extremely Desirable, 6= Desirable, 5= Slightly Desirable, 4= Acceptable, 3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 30: The Influence of Lactoferrin (LF) Application and Storage Time on Sensory Panelist Tenderness Scores[1] of Strip Loin Steaks

	Treatment[2]			
Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	6.23[de]	6.12[cde]	5.98[bcde]	5.72[abc]
21	5.95[bcde]	6.02 [bcde]	6.09[cde]	5.56[ab]
28	5.33[a]	6.29[e]	6.30[e]	6.00[bcde]
35	6.39[e]	6.22[de]	6.40[e]	5.79[abcd]

[abcde] LS means without a common superscript letter differ (P<0.0001).

[1] Tenderness: 8= Extremely Tender, 7= Very Tender, 6= Moderately Tender, 5= Slightly Tender, 4= Slightly Tough, 3=Moderately Tough, 2= Very Tough, 1= Extremely Tough.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 31: The Influence of Lactoferrin (LF) Application and Storage Time on Sensory Panelist Juiciness Scores[1] of Strip Lin Steaks

	Treatment[2]			
Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	5.98^{e}	5.72^{bcde}	5.74^{bcde}	5.75^{cde}
21	5.21^{a}	5.97^{e}	5.51^{abcd}	5.30^{ab}
28	5.40^{abc}	5.85^{de}	5.66^{abcde}	5.77^{cde}
35	6.05^{e}	5.68^{bcde}	5.91^{de}	5.78^{cde}

^{abcde} LS means without a common superscript letter differ (P<0.0001).

[1] Juiciness: 8= Extremely Juicy, 7= Very Juicy, 6= Moderately Juicy, 5= Slightly Juicy, 4= Slightly Dry, 3=Moderately Dry, 2= Very Dry, 1= Extremely Dry.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 32: The Influence of Lactoferrin (LF) Application and Storage Time on the Sensory Panelist Detection of Off Flavors [1] of Strip Loin Steaks

	Treatment[2]			
Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	1.29^{abcd}	1.17^{a}	1.17^{a}	1.56^{e}
21	1.23^{abc}	1.37^{bcde}	1.20^{ab}	1.29^{abcd}
28	1.40^{cde}	1.26^{abcd}	1.40^{cde}	1.36^{abcd}
35	1.34^{abcd}	1.31^{abcd}	1.27^{abcd}	1.43^{de}

^{ab} Within a row, LS means without a common superscript letter differ (P<0.0001).

[1] Off Flavors: 3= Strong, 2= Slightly Detectable, 1= Not Detectable.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 33: The Influence of Lactoferrin (LF) Application and Storage Time on Sensory Panelist Flavor Scores[1] of Strip Loin Steaks

	Treatment[2]			
Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	1.82	1.91	2.06	1.79
21	1.84	1.82	2.02	1.95
28	1.86	1.94	1.90	1.94
35	2.06	1.85	1.88	1.87

[ab] Within a row, LS means without a common superscript letter differ ($P<0.05$).

[1] Off Flavors: 3= Strong, 2= Slightly Detectable, 1= Not Detectable.

[2] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 34: The Influence of Lactoferrin (LF) Application on Oxidative Properties (TBA, mg MDA/kg sample) of Strip Loin Steaks at 1 and 14 d Retail Display

	Retail Display, d	
Treatment [1]	1	14
LF / LF	0.44 [a]	2.79 [b]
LF / NLF	0.44 [a]	2.82 [b]
NLF / LF	0.53 [a]	3.05 [b]
NLF / NLF	0.61 [a]	3.04 [b]

[ab] Within a row, LS means without a common superscript letter differ (P<0.05).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 35: The Influence of Lactoferrin (LF) Application and Storage Time on the Tenderness (WBS, kg) of USDA Select Strip Loin Steaks

	Treatment[1]			
Storage, d	LF / LF	LF / NLF	NLF / LF	NLF / NLF
14	3.32^{bc}	3.89^{g}	3.41^{cdef}	3.62^{defg}
21	3.66^{efg}	3.35^{cde}	3.71^{fg}	3.35^{cde}
28	3.53^{cdef}	3.39^{cde}	3.52^{cdef}	3.64^{efg}
35	3.03^{ab}	2.93^{a}	3.24^{abc}	3.35^{cde}

[abcdef] LS means without a common superscript letter differ (P<0.0001).

[1] LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to the subprimal and steak, respectively.

Table 36: Percentage of Acceptable Samples Remaining in Retail Case Within Lactoferrin Treatment each Day of Display

	Acceptable Product, %			
	Treatment[1]			
Retail Display, d	LF/LF	LF/NLF	NLF/LF	NLF/NLF
1	100	100	100	100
2	100	100	100	100
3	99.66	99.64	99.9	99.3
4	91	89	91	90
5	84	82	84	85
6	79	79	80	75
7	63	69	63	59
8	38	42	35	33
9	20	23	16	22
10	13	14	9	11
11	6	8	2	6
12	4	4	0	2
13	2	5	1	4
14	2	4	0	2

[1]LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to subprimal and steak, respectively.

Table 37: Estimated Carcass Values as Influenced by the Percentage of Case-Ready Packages Discarded as a Result of Inadequate Retail Shelf Life

	Discarded/Discounted Product %			
	Treatment[1]			
Retail display, d	LF/LF	LF/NLF	NLF/LF	NLF/NLF
1 (BASE)	$995.30	$995.30	$995.30	$995.30
2	$995.30	$995.30	$995.30	$995.30
3	$992.31	$992.31	$994.30	$988.33
4	$905.72	$895.77	$905.72	$895.77
5	$836.05	$865.91	$836.05	$816.15
6	$786.29	$796.24	$796.24	$746.48
7	$627.03	$686.76	$627.03	$567.32
8	$378.21	$418.03	$348.36	$308.54

[1]LF/LF, LF/NLF, NLF/LF, NLF/NLF represents whether lactoferrin was applied (LF) or not applied (NLF) to subprimal and steak, respectively.

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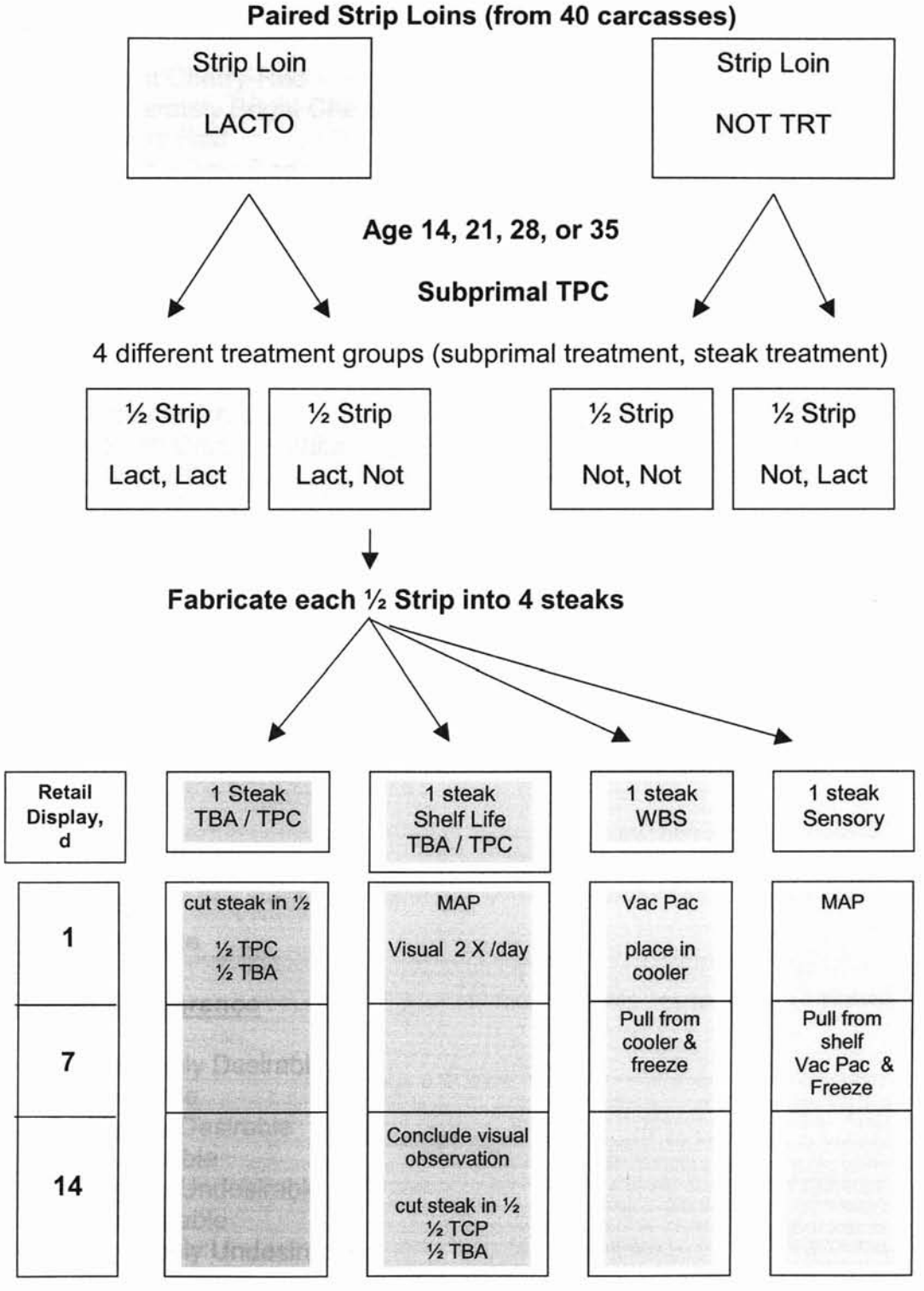
APPENDIX

Appendix A

SCHEMATIC OF EXPERIMENTAL DESIGN



Appendix B

VISUAL APPRAISAL GUIDELINES

Lean Color

8. Bright Cherry-Red
7. Moderately Bright Cherry
6. Cherry-Red
5. Slightly Dark Red
4. Moderately Dark Red or Brown
3. Dark Red or Brown
2. Very Dark Brown
1. Extremely Dark Brown

Fat Color

8. Creamy White
7. Mostly Creamy White
6. Slightly Tan
5. Tan
4. Slightly Brown
3. Moderately Brown
2. Brown or Slightly Green
1. Dark Brown or Green

Percent Discoloration

7. None
6. 1-10
5. 11-25
4. 26-50
3. 51-75
2. 76-99
1. Complete

Overall Appearance

7. Extremely Desirable
6. Desirable
5. Slightly Desirable
4. Acceptable
3. Slightly Undesirable
2. Undesirable
1. Extremely Undesirable

Appendix C

Bacteriological Analytical Manual (BAM)
US Food and Drug Administration
January 2001

Aerobic Plate Counts

Spiral Plate Method

The spiral plate count (SPLC) method for microorganisms in milk, foods, and cosmetics is an official method of the APHA (2) and the AOAC (3). In this method, a mechanical plater inoculates a rotating agar plate with liquid sample. The sample volume dispensed decreases as the dispensing stylus moves from the center to the edge of the rotating plate. The microbial concentration is determined by counting the colonies on a part of the petri dish where they are easily countable and dividing this count by the appropriate volume. One inoculation determines microbial densities between 500 and 500,000 microorganisms/ml. Additional dilutions may be made for suspected high microbial concentrations.

A.Equipment and materials

1.Spiral plater (Spiral Systems Instruments, Inc., 7830 Old Georgetown Road, Bethesda, MD 20814)
2.Spiral colony counter (Spiral Systems) with special grid for relating deposited sample volumes to specific portions of petri dishes
3.Vacuum trap for disposal of liquids (2-4 liter vacuum bottle to act as vacuum reservoir and vacuum source of 50-60 cm Hg)
4.Disposable micro beakers, 5 ml
5.Petri dishes, plastic or glass, 150 x 15 mm or 100 x 15 mm
6.Plate count agar (standard methods) (M124)
7.Calculator (optional), inexpensive electronic hand calculator is recommended
8.Polyethylene bags for storing prepared plates
9.Commercial sodium hypochlorite solution, about 5% NaOCl (bleach)
10.Sterile dilution water
11.Syringe, with Luer tip for obstructions in stylus; capacity not critical
12.Work area, storage space, refrigerator, thermometers, tally, incubator, as described for Conventional Plate Count Method, above.
13.Sodium hypochlorite solution (5.25%). Available commercially.

B.Preparation of agar plates.

Automatic dispenser with sterile delivery system is recommended to prepare agar plates. Agar volume dispensed into plates is reproducible and contamination rate is low compared to hand-pouring of agar in open laboratory. When possible, use laminar air flow hood along with automated dispenser. Pour same quantity of agar into all plates so that same height of agar will be presented to spiral plater stylus tip to maintain contact angle. Agar plates should be level during cooling.

The following method is suggested for prepouring agar plates: Use automatic dispenser or pour constant amount (about 15 ml/100 mm plate; 50 ml/150 mm plate) of sterile agar at 60-70°C into each petri dish. Let agar solidify on level surface with poured plates stacked no higher than 10 dishes. Place solidified agar plates in polyethylene bags, close with ties or heat-sealer, and store inverted at 0-4.4°C. Bring prepoured plates to room temperature before inoculation.

C.Preparation of samples.

As described in Chapter 1, select that part of sample with smallest amount of connective tissues or fat globules.

D.Description of spiral plater.

Spiral plater inoculates surface of prepared agar plate to permit enumeration of microorganisms in solutions containing between 500 and 500,000 microorganisms per ml. Operator with minimum training can inoculate 50 plates per h. Within range stated, dilution bottles or pipets and other auxiliary equipment are not required. Required bench space is minimal, and time to check instrument alignment is less than 2 min. Plater deposits decreasing amount of sample in Archimedean spiral on surface of prepoured agar plate. Volume of sample on any portion of plate is known. After incubation, colonies appear along line of spiral. If colonies on a portion of plate are sufficiently spaced from each other, count them on special grid which associates a calibrated volume with each area. Estimate number of microorganisms in sample by dividing number of colonies in a defined area by volume contained in same area. Studies have shown the method to be proficient not only with milk (4) but also with other foods (7,10).

Appendix C

E.Plating procedure

Check stylus tip angle daily and adjust if necessary. (Use vacuum to hold microscope cover slip against face of stylus tip; if cover slip plane is parallel at about I mm from surface of platform, tip is properly oriented). Liquids are moved through system by vacuum. Clean stylus tip by rinsing for 1 s with sodium hypochlorite solution followed by sterile dilution water for 1 s before sample introduction. This rinse procedure between processing of each sample minimizes cross-contamination. After rinsing, draw sample into tip of Teflon tubing by vacuum applied to 2-way valve. When tubing and syringe are filled with sample, close valve attached to syringe. Place agar plate on platform, place stylus tip on agar surface, and start motor. During inoculation, label petri plate lid. After agar has been inoculated, stylus lifts from agar surface and spiral plater automatically stops. Remove inoculated plate from platform and cover it. Move stylus back to starting position. Vacuum-rinse system with hypochlorite and water, and then introduce new sample. Invert plates and promptly place them in incubator for 48 ± 3 h at 35 ± 1°C.

F.Sterility controls

Check sterility of spiral plater for each series of samples by plating sterile dilution water. CAUTION: Prepoured plates should not be contaminated by a surface colony or be below room temperature (water can well-up from agar). They should not be excessively dry, as indicated by large wrinkles or glazed appearance. They should not have water droplets on surface of agar or differences greater than 2 mm in agar depth, and they should not be stored at 0-4.4°C for longer than I month. Reduced flow rate through tubing indicates obstructions or material in system. To clear obstructions, remove valve from syringe, insert hand-held syringe with Luer fitting containing water, and apply pressure. Use alcohol rinse to remove residual material adhering to walls of system. Dissolve accumulated residue with chromic acid. Rinse well after cleaning.

G.Counting grid

1.Description. Use same counting grid for both 100 and 150 mm petri dishes. A mask is supplied for use with 100 mm dishes. Counting grid is divided into 8 equal wedges; each wedge is divided by 4 arcs labeled l, 2, 3, and 4 from outside grid edge. Other lines within these arcs are added for ease of counting. A segment is the area between 2 arc lines within a wedge. Number of areas counted (e.g., 3) means number of segments counted within a wedge. Spiral plater deposits sample on agar plate in the same way each time. The grid relates colonies on spiral plate to the volume in which they were contained. When colonies are counted with grid, sample volume becomes greater as counting starts at outside edge of plate and proceeds toward center of plate.

2.Calibration. The volume of sample represented by various parts of the counting grid is shown in operator's manual that accompanies spiral plater. Grid area constants have been checked by the manufacturer and are accurate. To verify these values, prepare 11 bacterial concentrations in range of 106-103 cells/ml by making 1:1dilutions of bacterial suspension (use a nonspreader). Plate all Incubate both sets of plates for 48 ± 3 h at 35 ± 1°C. Calculate concentrations for each dilution. Count spiral plates over grid surface, using counting rule of 20 (described in H, below), and record number of colonies counted and grid area over which they were counted. Each spiral colony count for a particular grid area, divided by aerobic count/ml for corresponding spirally plated bacterial concentrations, indicates volume deposited on that particular grid area. Use the following formula:

$$\frac{31 + 30 \text{ colonies}}{0.0015 \text{ ml}} = 4.1 \times 10^4$$

To check total volume dispensed by spiral plater, weigh amount dispensed from stylus tip. Collect in tared 5 ml plastic beaker and weigh on analytical balance (± 0.2 mg).

H.Examination and reporting of spiral plate counts.

Counting rule of 20. After incubation, center spiral plate over grid by adjusting holding arms on viewer. Choose any wedge and begin counting colonies from outer edge of first segment toward center until 20 colonies have been counted. Complete by counting remaining colonies in segment where 20th colony occurs. In this counting procedure, numbers such as 3b, 4c (Fig. l) refer to area segments from outer edge of wedge to designated arc line. Any count irregularities in sample composition are controlled by counting the same segments in the opposite wedge and recording results. Two segments of each wedge were counted on opposite sides of plate with 31 and 30 colonies, respectively. The sample volume contained in the darkened segments is 0.0015 ml. To estimate number of microorganisms, divide count by volume contained in all segments counted. See example under Fig. l.

If 20 CFU are not within the 4 segments of the wedge, count CFU on entire plate. If the number of colonies exceeds 75 in second, third, or fourth segment, which also contains the 20th colony, the estimated number of microorganisms will generally be low because of coincidence error associated with crowding of colonies. In this

case, count each circumferentially adjacent segment in all 8 wedges, counting at least 50 colonies, e.g., if the first 2 segments of a wedge contain 19 colonies and the third segment contains the 20th and 76th (or more), count colonies in all circumferentially adjacent first and second segments in all 8 wedges. Calculate contained volume in counted segments of wedges and divide into number of colonies.

When fewer than 20 colonies are counted on the total plate, report results as "less than 500 estimated SPLC per ml." If colony count exceeds 75 in first segment of wedge, report results as "greater than 500,000 estimated SPLC per ml." Do not count spiral plates with irregular distribution of colonies caused by dispensing errors. Report results of such plates as laboratory accident (LA). If spreader covers entire plate, discard plate. If spreader covers half of plate area, count only those colonies that are well distributed in spreader-free areas.

Compute SPLC unless restricted by detection of inhibitory substances in sample, excessive spreader growth, or laboratory accidents. Round off counts as described in I-D, above. Report counts as SPLC or estimated SPLC per ml.

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Hypertext Source: Bacteriological Analytical Manual, Edition 8, Revision A, 1998. Chapter 3.
*Authors:Larry J. Maturin and James T. Peeler

Appendix D

SENSORY BALLOT

BOOTH # _____ DATE: _____ TIME: _____ AM / PM

Sample	Tenderness	Juiciness	Cooked Beef Flavor	Off Flavor	Overall Acceptability
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					

Tenderness
8 Extremely Tender
7 Very Tender
6 Moderately Tender
5 Slightly Tender
4 Slightly Tough
3 Moderately Tough
2 Very Tough
1 Extremely Tough

Juiciness
8 Extremely Juicy
7 Very Juicy
6 Moderately Juicy
5 Slightly Juicy
4 Slightly Dry
3 Moderately Dry
2 Very Dry
1 Extremely Dry

Overall Acceptability
7 Extremely Desirable
6 Desirable
5 Slightly Desirable
4 Acceptable
3 Slightly Undesirable
2 Undesirable
1 Extremely Undesirable

Cooked Beef Flavor
3 Strong
2 Slightly Detectable
1 Not Detectable

Off Flavor
3 Strong
2 Slightly Detectable
1 Not Detectable

Appendix E

Microsomal Lipid Peroxidation

John A. Buege and Steven D. Aust

Methods in Enzymology 52:306

The Thiobarbituric Acid Assay

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm.[27]

Reagent

Stock TCA–TBA–HCl reagent: 15% w/v trichloroacetic acid: 0.375% w/v thiobarbituric acid: 0.25 *N* hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid.

Procedure. Combine 1.0 ml of biological sample (0.1–2.0 mg of membrane protein or 0.1–0.2 μmol of lipid phosphate) with 2.0 ml of TCA–TBA–HCl and mix thoroughly. The solution is heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate is removed by centrifugation at 1000 *g* for 10 min. The absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid. The malondialdehyde concentration of the sample can be calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.[28]

Appendix F

Assay of lipid oxidation in muscle samples
Modified from J. A. Buege and S. D. Aust, 1978.
Methods in Enzymol. 52:302, AP

1. TBA assay

Reagent:

1. TCA/TBA stock solution: 15% TCA (w/v) and 20 mM TBA (MW 144.15) reagent in DW. Dissolve 2.88 g TBA in warm DDW first, add TCA (150 g) and then Add DW to the mark (1L).
2. BHA: Make 10% stock solution by dissolving in 90% ethanol.
3. TEP standard :1 x 10^{-3} M 1,1,3,3-tetra-ethoxypropane in DW. This solution can be kept for about a week if stored in the refrigerator and diluted as needed. (MW 220.31, 95% purity, d= 0.918). Dilute 0.5 ml TEP with 499.5 ml DW, and dilute the resulting solution 1: 2.96 (TEP solution: DW) with DW.

Procedure

Grind meat twice (through a 3-mm plate) or remove surface tissue and dice before use. Place the 10 g meat sample blender cup with 3 vol (30 mL) of DW, and then homogenize for 10-15 sec (or homogenize for 10-15 sec using a polytron at speed 7-8). Centrifuge at 3,000 RPM (1,850 g) fro 10 min at 4°C. Take 2 ml of the homogenate, combine with 4 ml of the TCA/TBA reagent, 100 □l BHA, mix thoroughly and then heat the solution for 15 min in a boiling water bath. Cool for 10 min in cold water, vortex thoroughly, and centrifuge at 3,000 RPM (1,850 g) for 10 min. Read the absorbance of the supernatant at 531 nm against a blank that contains all the reagents minus sample. Construct TBA standard curve using TEP.

Malonaldehyde standard curves ($CHO-CH_2-CHO$, MW 72.0)

1. Make work solutions by diluting 1x 10^{-3} M TEP standard solution (72 ppm MDA std). [Dilute 0.5 ml TEP with 499.5 ml DW, and dilute the resulting solution 1 : 2.96 (TEP solution : DW) with DW].
2. Take 0, 5, 10, 20, 30, 40, and 50 □l of 1x 10^{-3} M TEP standard solution into each test tube and add DW to make 1 ml solution.
3. Add 2 ml TBA/TCA to each work solution and mix well.
4. Heat the solution for 15 min in a boiling water bath, cool, vortex thoroughly.
5. Read the optical density of the standard against a blank at a wavelength 531 nm.

Appendix G

BOXED BEEF YIELD VALUE CALCULATOR

OKLAHOMA STATE UNIVERSITY BOXED BEEF YIELD VALUE CALCULATOR 1998

These data updated on 04/23/01

	INPUTS	TRIM LEVEL	COMMOD.	0.25 INCH
CARCASS WEIGHT LBS	816	CALC ULATED LIVE WT	1280	1280
QUALITY GRADE (1-5)	4	GROSS CARC VALUE	$975.45	$995.30
YIELD GRADE (1.0 TO 4.9)	2.90	EST DROP CREDIT	$102.40	$102.40
DROP CREDIT $ / CWT	$8.00	GROSS LIVE VALUE	$1,077.85	$1,097.70
ESTIMATED DRESS %	63.75	NET CARCASS $/CWT	$119.64	$121.34
KILL-FAB COST EST. COMOD.	$97.00	NET LIVE $/CWT	$76.27	$77.35
KILL-FAB COST EST. .25 INCH	$103.00	US SELECT		CLOSE PREM.
CATTLE FREIGHT $ / CWT	$0.36	YIELD GRADE 2		$13.85
	RECOVERY AS A % OF HOT WT*		98.06%	97.47%
	PERCENT BOX BEEF YIELD		69.51%	65.91%

Kill and Fabrication Costs	Commodity	Close
Yield Grade 1	$94.00	$100.00
Yield Grade 2	$97.00	$103.00
Yield Grade 3	$100.00	$106.00
Yield Grade 4	$116.00	$124.00

* Recovery as a percent of hot carcass weight represents the error in our regression equations in the prediction of the sum of the box cuts. It does not represent cooler shrink or cutting losses. In some cases recovery can exceed 100 percent. This is the reason for restricting the use of these equations as cited below.

IMPORTANT NOTICE: THE DATA USED IN MAKING THESE ESTIMATES WERE OBTAINED FROM CUTTING TESTS IN A COMMERCIAL PACKING PLANT. 453 STEERS AND 120 HEIFERS WERE FABRICATED. THE CARCASSES WEIGHED 555 TO 1008 POUNDS. FAT THICKNESS RANGED FROM 0.08 TO 1.28 INCHES. RIBEYE AREA RANGED FROM 9.3 TO 18.9 sq.in. THE TEST CARCASSES GRADED 60.2% U.S. CHOICE AND 39.8% U.S. SELECT.

SUGGESTED USE RANGE IS 650 TO 875 POUND CARCASSES AND YIELD GRADES BETWEEN 1.0 AND 4.5.

DEVELOPED AT OKLAHOMA STATE UNIVERSITY BY GLEN DOLEZAL, DONALD GILL AND TOM GARDNER

Appendix H

BOXED BEEF VALUE ON CARCASS BASIS

The data shown on this sheet were for a carcass with the folowing specifications:

	C-Weight	Y- Grade	Q-Grade
	816	2.90	S SELECT

These data updated on 04/23/01

	SIDE BASIS		CARCASS BASIS				CLOSE	COMMOD
	WEIGHT	WEIGHT	WEIGHT	WEIGHT	PRICE	PRICE	PRODUCT	PRODUCT
BOXED BEEF CUTS	CLOSE	COMMOD	CLOSE	COMMOD	CLOSE	COMMOD	VALUE	VALUE
112A RIBEYE <11 lbs	13.70	13.70	27.41	27.41	$420.00	$420.00	$120.58	$120.58
112A RIBEYE 11> lbs	13.70	13.70	27.41	27.41	$440.00	$440.00		
114 SH CLOD	21.34	23.22	42.68	46.43	$130.00	$118.00	$55.48	$54.79
116A CHUCK ROLL	31.76	34.67	63.52	69.35	$128.00	$132.00	$81.31	$91.54
120 BRISKET	10.69	12.73	21.38	25.46	$130.00	$102.00	$27.80	$25.97
167 KNUCKLE	11.14	11.78	22.27	23.55	$159.00	$147.00	$35.41	$34.62
168 INSIDE RND	22.53	24.43	45.07	48.85	$150.00	$140.00	$67.60	$68.39
170 GOOSENECK	29.54	30.89	59.08	61.78	$154.00	$127.00	$90.98	$78.46
180 STRIP LOIN <12 lbs	13.19	15.23	26.39	30.46	$440.00	$340.00	$116.11	$103.58
180 STRIP LOIN 12-13.9 #	13.19	15.23	26.39	30.46	$440.00	$340.00		
180 STRIP LOIN 14> lbs	13.19	15.23	26.39	30.46	$440.00	$340.00		
184 TOP BUTT <12 lbs	12.47	13.97	24.95	27.95	$230.00	$218.00	$57.38	$60.93
184 TOP BUTT 12> lbs	12.47	13.97	24.95	27.95	$230.00	$218.00		
185A BOT SRLN FLAP	3.94	3.94	7.88	7.88	$287.00	$287.00	$22.62	$22.62
185B BOT SRLN BALL TIP <2	2.51	2.51	5.02	5.02	$268.00	$268.00	$13.46	$13.46
185B BOT SRLN BALL TIP 2>	2.51	2.51	5.02	5.02	$268.00	$268.00		
185C BOT SRLN TRITIP	2.92	3.39	5.84	6.77	$295.00	$165.00	$17.23	$11.17
189A TENDERLOIN <5 lbs	6.35	6.35	12.70	12.70	$680.00	$680.00	$89.56	$89.56
189A TENDERLOIN 5> lbs	6.35	6.35	12.70	12.70	$705.00	$705.00		
193 FLANK STEAK	2.04	2.04	4.08	4.08	$390.00	$390.00	$15.90	$15.90
INSIDE SKIRT	4.69	4.69	9.37	9.37	$245.00	$245.00	$22.96	$22.96
CAP & WEDGE MEAT	13.52	13.52	27.04	27.04	$135.00	$135.00	$36.50	$36.50
BACK RIBS	7.02	7.02	14.03	14.03	$79.00	$79.00	$11.08	$11.08
80% LEAN TRIM	36.30	36.30	72.61	72.61	$110.00	$110.00	$79.87	$79.87
50% LEAN TRIM	23.24	23.24	46.48	46.48	$72.00	$72.00	$33.46	$33.46
			CLOSE	COMMOD			$995.30	$975.45
EDIBLE TALLOW	73.37	61.07	146.74	122.14				
BONE	55.42	55.42	110.84	110.84				
TOTAL PRODUCT POUNDS	397.69	400.10	795.37	800.19				
PERCENT BOX BEEF YIELD			65.91%	69.51%				

Appendix I

BOXED BEEF PRICES

These data updated on 04/23/01

BOXED BEEF CUTS	PRICES FOR COMMODITY TRIM PRODUCTS					PRICES FOR CLOSELY TRIMED PRODUCTS				
(GRADE—>>)	PRIME	PREM CH	CHOICE	SELECT	NO ROLL	PRIME	PREM CH	CHOICE	SELECT	NO ROLL
112A RIBEYE <11 lbs	$555.00	$500.00	$490.00	$420.00	$410.00	$555.00	$500.00	$490.00	$420.00	$410.00
112A RIBEYE 11> lbs	$550.00	$525.00	$515.00	$440.00	$420.00	$550.00	$525.00	$515.00	$440.00	$420.00
114 SH CLOD	$118.00	$118.00	$118.00	$118.00	$117.00	$130.00	$130.00	$130.00	$130.00	$129.00
116A CHUCK ROLL	$132.00	$124.00	$124.00	$132.00	$131.00	$170.00	$128.00	$128.00	$128.00	$127.00
120 BRISKET	$109.00	$109.00	$99.00	$102.00	$102.00	$137.00	$137.00	$127.00	$130.00	$130.00
167 KNUCKLE	$147.00	$147.00	$147.00	$147.00	$147.00	$159.00	$159.00	$159.00	$159.00	$159.00
168 INSIDE RND	$159.00	$159.00	$149.00	$140.00	$137.00	$169.00	$169.00	$159.00	$150.00	$147.00
170 GOOSENECK	$127.00	$127.00	$127.00	$127.00	$126.00	$154.00	$154.00	$154.00	$154.00	$153.00
180 STRIP LOIN <12 lbs	$560.00	$415.00	$390.00	$340.00	$325.00	$750.00	$550.00	$525.00	$440.00	$425.00
180 STRIP LOIN 12-13.9 #	$560.00	$415.00	$390.00	$340.00	$325.00	$750.00	$550.00	$525.00	$440.00	$425.00
180 STRIP LOIN 14> lbs	$560.00	$415.00	$390.00	$340.00	$325.00	$750.00	$550.00	$525.00	$440.00	$425.00
184 TOP BUTT <12 lbs	$310.00	$269.00	$264.00	$218.00	$213.00	$344.00	$295.00	$290.00	$230.00	$225.00
184 TOP BUTT 12> lbs	$310.00	$269.00	$264.00	$218.00	$213.00	$344.00	$295.00	$290.00	$230.00	$225.00
185A BOT SRLN FLAP	$287.00	$287.00	$287.00	$287.00	$287.00	$287.00	$287.00	$287.00	$287.00	$287.00
185B BOT SRLN BALL TIP	$268.00	$268.00	$268.00	$268.00	$263.00	$268.00	$268.00	$268.00	$268.00	$263.00
185B BOT SRLN BALL TIP	$268.00	$268.00	$268.00	$268.00	$263.00	$268.00	$268.00	$268.00	$268.00	$263.00
185C BOT SRLN TRITIP	$265.00	$265.00	$260.00	$165.00	$160.00	$295.00	$295.00	$295.00	$295.00	$173.00
189A TENDERLOIN <5 lbs	$1,010.00	$720.00	$710.00	$680.00	$680.00	$1,010.00	$720.00	$710.00	$680.00	$680.00
189A TENDERLOIN 5> lbs	$1,010.00	$800.00	$790.00	$705.00	$705.00	$1,010.00	$800.00	$790.00	$705.00	$705.00
193 FLANK STEAK	$434.00	$434.00	$424.00	$390.00	$390.00	$434.00	$434.00	$424.00	$390.00	$390.00
INSIDE SKIRT	$255.00	$255.00	$245.00	$245.00	$245.00	$255.00	$255.00	$245.00	$245.00	$245.00
CAP & WEDGE MEAT	$135.00	$135.00	$135.00	$135.00	$135.00	$135.00	$135.00	$135.00	$135.00	$135.00
BACK RIBS	$89.00	$89.00	$79.00	$79.00	$79.00	$89.00	$89.00	$79.00	$79.00	$79.00
80% LEAN TRIM	$110.00	$110.00	$110.00	$110.00	$110.00	$110.00	$110.00	$110.00	$110.00	$110.00
50% LEAN TRIM	$72.00	$72.00	$72.00	$72.00	$72.00	$72.00	$72.00	$72.00	$72.00	$72.00

VITA

Laura Lee Locke

Candidate for the Degree of

Master of Science

Thesis: THE INFLUENCE OF ACTIVATED LACTOFERRIN AS A MICROBIAL BLOCKING AGENT ON SENSORY AND SHELF LIFE CHARACTERISTICS OF CASE-READY FRESH BEEF

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Biographical:

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Experience: Raised in New Castle, Ohio on a family farm with parents who placed emphasis upon responsibility and leadership through various endeavors. Employed by Oklahoma State University, Department of Animal Science as an undergraduate 1997 – 2000, USDA Meat Grading and Certification branch as an intern 1999, and Oklahoma State University as a graduate research assistant, 2000 to present.

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