IMPROVING SHELF-LIFE OF BISON STEAKS TREATED WITH OREGANO AND ROSEMARY EXTRACTS

By

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ABSTRACT

The effects of plant extracts (non-enhanced, 0.05 % rosemary and 0.08% oregano extracts in the final product at a 10% pump level) on shelf life traits and consumer acceptability of bison striploin steaks were studied. Oregano steaks presented lower oxygen consumption and higher metmyoglobin reducing activity (MRA), decreased lipid oxidation and provided a stable red colour with less discolouration during the retail display period than the control and rosemary steaks ($P < 0.05$). Consumers indicated that treated steaks under study were not significantly different to the control group ($P > 0.05$) on palatability attributes and acceptability. However, rosemary injected steaks were more desirable and palatable than oregano counterpart ($P < 0.05$). In conclusion, plant extracts, particularly oregano, can considerably improve colour stability of bison steaks due to its antioxidants properties and ability to increase MRA capacity in the bison meat without posing any negative impact on its sensory attributes.

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DEDICATION

I dedicate this thesis to my mom, Vandana Sood and dad, Mr. Sandeep Sood for always believing in me and supporting me in every possible way.

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ABBREVIATIONS USED

AMSA	American Meat Science Association
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BL	Blast Chilling
COMb	Carboxymyoglobin
DFD	Dark, firm and dry
DMb	Deoxymyoglobin
ETC	Elevated temperature conditioning
HNE	2-hydroxy-2-nonenal
LVES	Low voltage electrical stimulation
Mb	Myoglobin
MMb	Metmyoglobin
MRA	Metmyoglobin reducing activity
NADH	Nicotinamide adenine dinucleotide
OC	Oxygen consumption
OMb	Oxymyoglobin
PG	Propyl gallate
PSE	Pale, soft, and exudative
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tetrebutylated hydroxyquinone

TCA	Trichloroacetic acid

CHAPTER 1: INTRODUCTION

Bison meat is more tender (Koch, Jung, Crouse, Varel, & Cundiff, 1995) and nutritionally denser than beef with a high proportion of protein (Galbraith et al., 2006; Marchello, Slanger, Hadley, Milne, & Driskell, 1998; Marchello & Driskell, 2001) and greater concentration of iron, zinc and essential fatty acids (Canadian Bison Association, 2018). Despite these advantages, the bison industry in Canada is still small but growing and trying to position its meat products in the Canadian red-meat marketplace (Steiner, Gao, & Unterschultz, 2010); particularly, in the market for discriminating consumers (natural and low-fat meat products), who are looking for alternatives to traditional red meats without sacrificing an excellent eating experience (SMA, 2000).

Fresh meat colour is one of the main sensory characteristics used by consumers to select or reject meat cuts at the retail level and serves as an indicator of freshness and wholesomeness of the meat. However, bison meat colour has been shown to be particularly unstable under aerobic packaging (Janz & Aalhus, 2006; Pietrasik, Dhanda, Shand, & Pegg, 2006), which may limit expansion of market opportunities because any deviation from the normal colour (e.g. cherry red), particularly browning, is indicative of poor functionality and possible spoilage (Faustman & Cassens, 1990). In order to expand its fresh market, bison industry needs to address the dark colour of bison meat (Koch et al., 1995) and its rapid discolouration problems (Dhanda, Pegg, Janz, Aalhus, & Shand, 2002).

According to Gunders (2012), about 22% of total meat that comes into the North American market for consumption is lost in different steps of the food supply chain which causes a major setback to the industry economically. In addition to that, discolouration discounts at retail also

have a significant economic impact, calculated to be several hundred million per year in the U.S. beef industry alone (Gunders 2012). The relatively small bison industry cannot absorb extensive losses like these. Hence, the underlying cause and mitigation strategies to control early retail browning must be in place before expansion to fresh meat sales can be considered.

Some technologies have been applied on bison meat with the purpose of mitigating the rapid discolouration and off-flavour development, such as blast chilling, spray chilling, elevated temperature conditioning, moisture enhancement, electrical stimulation and marination (Dhanda et al., 2002; Janz & Aalhus, 2006; Janz, Aalhus, & Price, 2001; Janz, Aalhus, Price, & Schaefer, 2000; Pietrasik et al., 2006) which have improved, in some cases, organoleptic characteristics of high-value cuts (i.e. ribeye), but have had limited, or no significant effect on the development or stability of bison meat colour.

Another worthy cause of deterioration in meat quality during storage and processing is lipid oxidation (Prado et al., 2015), which affects fatty acids, particularly polyunsaturated fatty acids (Allen & Foegeding, 1981; Gray, 1978). The primary and secondary oxidation products modify flavour and texture, decrease nutritional quality and also incur negative changes in colour (Buckley, Morrissey, & Gray,1995) which consequently limit meat shelf life.

The known chemistry associated with oxidation of lipids and myoglobin provides a fundamental basis by which these reactions can exacerbate each other. The reactions responsible for myoglobin (Mb) and lipid oxidation generate products that can act mutually to accelerate oxidation (Faustman, Sun, Mancini, & Suman, 2010) which can further deteriorate the colour stability along with other attributes. These oxidative changes are controlled by naturally present antioxidants such as carnosine (Solarska, Lewińska, Karowicz-Bilińska, & Bartosz, 2010), lactate (Kim et al., 2009), pyruvate (Zlotnik et al., 2008) which are often lost during conversion

of muscle to meat, processing, and storage. Thus, using antioxidants in the final product could minimize oxidative deterioration during storage and consequently increase the shelf-life of the product.

Antioxidants are compounds that have the ability to delay, retard or prevent lipid oxidation processes (Shahidi, Janitha, & Wanasundara, 1992) by stabilizing free radicals by donating hydrogen (H) to free radicals, or accepting electrons from free radicals to form a complex (Maisuthisakul, Suttajit, & Pongsawatmanit, 2007). Although synthetic antioxidants are widely used in industrial processing, some of them such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) could cause carcinogenic effects in living organisms (Karpińska, Borowski, & Danowska-Oziewicz, 2001). Therefore, natural antioxidants of plant origin have been introduced to improve the lipid stability and enhance the sensory properties of food. The antioxidant properties of natural antioxidants of plant origin are mainly attributed to their phenolic contents. Thus, their antioxidant action is similar to synthetic phenolic antioxidants (Durling et al., 2007). They can retard or inhibit the oxidation of meat by preventing the oxidizing chain reactions (Velioglu, Mazza, Gao, & Oomah, 1998), one of the major causes of chemical spoilage, preventing rancidity and/or deterioration of the nutritional quality, colour, flavour, and texture (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002).

Several plant extracts from diverse sources such as fruits, tea, spices and aromatic plants have been used as natural food antioxidants. Although, several authors have investigated the antioxidant potential of many plant extracts in different meat products, the effect of selected natural herbal extracts, such as oregano and rosemary, on the quality characteristics of meat is still limited and has not yet been studied in bison meat.

Oxidative mechanisms investigated in beef could be useful to understand and increase the limited existing scientific information about bison. However, even though bison and beef cattle are both ruminant meat species, they could have species-specific variation in meat due to the application of natural antioxidants on metmyoglobin formation, colour properties, biochemical changes and lipid oxidation and those characteristics have not yet been evaluated in bison. Additionally, the addition of these extract to fresh meat might cause change in palatability attributes, flavour profile and overall acceptability of bison meat which needs to be evaluated in order to make any industrial applications of the results found in this study.

Consequently, the main objectives of this thesis are:

1. To study the effect of oregano and rosemary extracts on the colour stability of bison strip loins in retail display conditions;

2. To examine the effect of oregano and rosemary extract on lipid oxidation of bison strip loin steaks; and

3.To determine the influence of oregano and rosemary extract on the final product palatability and acceptability.

CHAPTER 2: LITERATURE REVIEW

2.1 Meat color

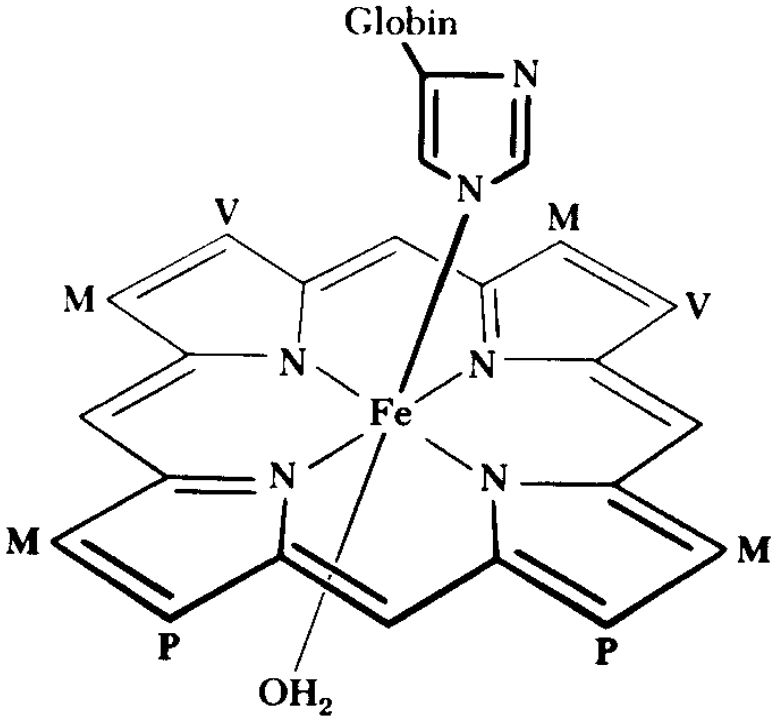
Visual appearance and palatability of meat products are the two key factors that impact consumers' preference for buying a meat product (Naumann, McBee, & Brady, 1957). Palatability attributes such as flavor, tenderness and juiciness cannot be predisposed at the point of sale, therefore color and visual appearance are the major forces that drive the purchasing decisions of fresh meat of the customer (Carpenter, Cornforth, & Whittier, 2001; Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2001). Visual appeal and color work as an indicator of freshness and wholesomeness (Mancini & Hunt, 2005) and give an apprehension for the product to have a high-quality eating experience and a long shelf-life. Any deviation from cherry-red colour appearance is indicative of poor color functionality and possible spoilage, and discolored meat cuts are often sold at discounted prices or ground to lower-value products such as ground meat, and if discoloration (early browning) is extensive, the product will be discarded (Suman, Hunt, Nair, & Rentfrow, 2014). In reality there is not a well-established correlation between fresh meat color and palatability (Troy & Kerry, 2010).

Meat color is one of the most crucial factors in the field of meat quality study. Myoglobin (Mb) is the primary protein responsible for pigment in muscle, contributing 80-90% of total pigment in meat (Aberle, Forrest, Gerrard, & Mills, 2011). Two other heme proteins namely, hemoglobin and cytochrome C may also contribute to pigment in muscle, but to a lesser extent (Mancini & Hunt, 2005).

2.1.1 Myoglobin Structure

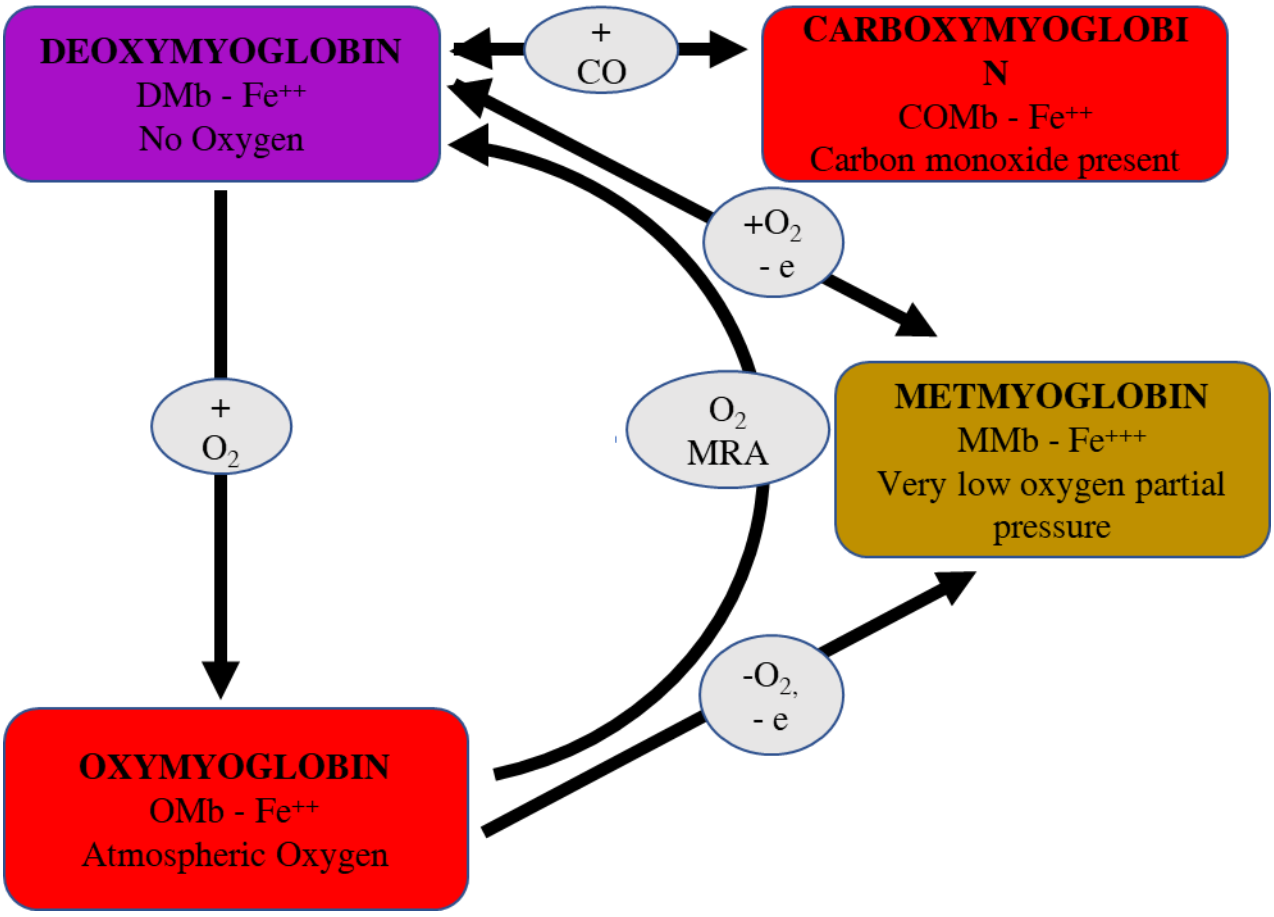
Myoglobin is a water-soluble protein containing 8 α-helices (A–H) that form a coiled structure that surrounds a monomeric heme prosthetic group which has a centrally located iron atom with six coordination binding sites (Figure 2.1).

Figure 2.1. Simplified structure of Myoglobin; Adapted from Seideman et al., (1984)



The iron atom can exist in the reduced ferrous (Fe^{2+}) state or the oxidized ferric (Fe^{3+}) state. Four of those sites are bound to nitrogen, the fifth site is bound to proximal histidine-93 which connects the heme to the globin chain, leaving the sixth binding site available to bind ligands reversibly. The ligand present and the valency of iron. dictate muscle color. In fresh meat, four chemical states of myoglobin can exist: deoxymyoglobin (DMb), oxymyoglobin (OMb), metmyoglobin (MMb) and carboxymyoglobin (COMb) as described in Figure 2.2.

Figure 2.2. Visual Conversion Pathways for Myoglobin Forms in Fresh Meats (Adapted from: Mancini & Hunt, 2005).



Beef myoglobin is comprised of approximately 153 amino acids and contains eight alpha-helices that are linked by non-helical sections (Suman & Joseph, 2013). Structurally, myoglobin is comprised of a globin moiety in addition to a heme protein within a heme prosthetic group. As pointed out by Suman and Joseph (2013) the presence of conjugated double bonds in the heme group gives myoglobin the ability to absorb light and provide pigmentation. Similarly, the eight globin helices form a protective structure around the heme group and offer some protection from oxidation and other external stressors.

A study conducted by Joseph et al. (2010) characterized the Mb structure in Bison meat and found that Bison Mb shared 100% amino acid sequence similarity with beef. Since there is limited literature from studies conducted on Bison meat, work cited for beef will be considered because of close resemblance in the chemistry of the two-meat species.

2.1.2. Myoglobin chemistry

Myoglobin (Mb) exists in different forms depending on the redox state of the iron atom of its heme part. In the native Deoxymyoglobin (DMb) reduced ferrous (Fe^{2+}) iron state, meat appears dark purplish-red or purplish-pink. Very low oxygen tension (<1.4 mm of Hg) (Brooks, 1935) within vacuum package or interior of the meat is required to maintain Mb in its DMb form. The DMb state has no ligand attached in its 6th co-ordination position and converts to OMb when it is exposed to oxygen and incorporates diatomic oxygen in its 6th site (Lindahl, 2005). The oxygenation of Mb at an atmospheric oxygen level produces the bright, red, "bloomed" colour of fresh meat via formation of OMb. No change in iron's valence occurs during oxygenation although the 6^{th} coordination site is now occupied by diatomic oxygen (Mancini & Hunt, 2005). Similarly, the exposure of myoglobin to carbon monoxide, even in the presence of oxygen, results in the formation of a remarkably stable bond with carbon monoxide (Sørheim, Nissen, & Nesbakken, 1999) and the formation of COMb. The heme iron's affinity for carbon monoxide allows for the formation of an extremely stable pigment when compared to OMb, which is markedly less stable (Mancini & Hunt, 2005). Metmyoglobin (MMb) is the oxidized tan to brown colored form of Mb formed by oxidation of the pigment from its ferrous (Fe^{2+}) to its ferric (Fe^{3+}) iron state under conditions of low oxygen partial pressure with water as the ligand at 6^{th} position of iron. Typically, MMb forms easily at low concentrations of oxygen (7mm Hg or

about 1 to 2% oxygen; AMSA, 2012) and decreases with a declining partial oxygen pressure up to 30mm Hg (Lindahl, 2005).

In meat, the three states of myoglobin exist simultaneously, and MMb formation tends to initiate between the outer OMb and interior DMb layer (AMSA, 2012). Although discoloration is often referred to as the amount of surface area covered by metmyoglobin, subsurface myoglobin forms also play a role in product appearance. This is because metmyoglobin beneath the surface (located between superficial OMb and interior DMb) gradually thickens and moves towards the surface. Low MMb formation in fresh meat occurs when oxygen is completely excluded from the environment or present at high levels (Faustman & Cassens, 1990). High oxygen partial pressures (above 21% oxygen; atmospheric O_2) have been shown to slow MMb formation (Jeremiah & Gibson, 2001).

Color deterioration (MMb formation) is not perceptible until greater than 30% of the pigments are in the oxidized state but becomes unacceptable colour when over 60% of the myoglobin is in the metmyoglobin form (Seideman, Cross, Smith and Durland, 1984).

2.1.3. Factors influencing fresh meat color

Literature illustrates that a multitude of factors (endogenous and exogenous) contribute to meat color stability and biochemical stability from the live animal to post-mortem. It is important to understand how each factor contributes to the stability of meat so that they could be manipulated to control the stability of meat color to be able to maximize display life of meat. The scientific principles behind these endogenous and exogenous factors have been exploited to develop strategies in meat processing and animal production to minimize discoloration (Suman & Joseph, 2013).

Extrinsic factors such as animal genetics, gender, age, diet energy density, time-on-feed, seasonality, ante mortem stress, carcass weight, many post-mortem conditions (method of immobilization, several cooler parameters affecting rate of chilling, carcass spacing and alignment, scalding and singing, criteria for carcass electrical stimulation, and application of antimicrobial interventions), post-mortem processing and packaging methods, time and temperature of storage, extent of exposure to oxygen and the number of cycles meat goes through the color cycle, and especially post-mortem age of the product usually influence meat color (AMSA, 2012).

With increase in age, myoglobin content increases (Aberle, Forrest, Gerrard, & Mills, 2001). The pale muscles of veal carcasses are indicative of the fact that the muscles of immature animals have a lower myoglobin content than those of more mature individuals resulting in darker muscle colour to the latter. Sex also has an influence in myoglobin concentration, with intact males having higher concentrations of myoglobin than that of their castrated or female counterparts (Aberle et al., 2001). In general, beef and lamb have more myoglobin than pork, veal, fish, and poultry.

Temperature also plays a part in colour stability because as higher temperatures favour increase in oxygen consumption, microbial growth, and lipid oxidation rate, all of which effect discoloration in meat. Inversely, temperature decreases will result in the delayed onset of discoloration; therefore, slowing the oxygenation of myoglobin (Seideman et al., 1984). This occurs because the low temperature will promote the penetration of oxygen into the meat's surface and will increase the oxygen solubility, helping to maintain the OMb state (Renerre, 2000). Hood and Riordan (1973) reported that discoloration in beef was 2-5 times higher when stored in 10°C than when stored in 0°C after four days of storage.

In addition, these exogenous factors influence the i**ntrinsic factors** such as pH, muscle type, areas within a muscle, muscle fiber composition, myoglobin concentration, disruption of various subcellular components related to meat color chemistry, water holding capacity, microbial load, and temperature history affects two critical factors tremendously, meat's use of oxygen and meat's ability to reduce MMb.

The rate of post-mortem pH decline and the ultimate pH in post-rigor muscle both affect meat color stability. Ultimate pH in post rigor muscle of normal meat is approximately 5.4-5.8. An abnormal rate of pH decline can lead to one of two color defects. The lower pH values cause the muscle structure (fibrils) to be more "open" and heme protein to be exposed causing scattering of light and thus, appear paler in color (Seideman et al., 1984; Walters, 1975). This is commonly referred to as pale, soft, and exudative (PSE; Greene, Hsin, & Zipser, 1971) meat which occurs mainly in pork but can also occur in other species such as beef, lamb, and poultry. The opposite condition of PSE is known as DFD (dark, firm and dry) in beef and pork and is caused by a high ultimate pH (Seideman et al., 1984).

Color of meat also differs with muscle and fiber type. Some muscles are more consistent in color than others during post-mortem storage. Muscles used in locomotion generally have a higher myoglobin concentration than support muscles due to the high oxygen requirement for energy in the muscle (Seideman et al., 1984). Muscles that have a high proportion of red fibers will appear to be dark red (Aberle et al., 2001). This is due to the fact that red muscle fibers predominantly use aerobic metabolism in which the oxygen is transported by myoglobin, leading to a higher concentration of myoglobin in these fibers than in white muscle fibers which function more on anaerobic metabolism (Seideman et al., 1984). Color of meat also differs with different species as for example; color of beef is redder than pork.

Many studies (Arihara, Cassens, Greaser, Luchansky, & Mozdziak, 1995; Madhavi & Carpenter; Renerre & Labas, 1987; Sammel, Hunt, Kropf, Hachmeister, & Johnson, 2002) have shown the relationship between oxygen consumption and some partially undefined system named metmyoglobin reductase (MMR) as a factor in deciding meat color post-mortem.

The ability of muscle to maintain the heme iron in a reduced state—which is critical to avoid the formation of MMb—is referred to as MRA. In living muscle, myoglobin is always in reduced form and actively transports and stores oxygen (Sammel et al., 2002). The oxidized form of myoglobin is MMb which is normally reduced by MMb reducing system (MRS) in living cell. The muscle's ability to reduce metmyoglobin is affected by several factors, but primarily by the muscle's oxygen scavenging ability and supply of reducing equivalents (i.e. NADH). Muscles which effectively reduce MMb to DMb are able to abstract an electron from NADH for the ferric heme iron. Thus, maintaining a muscle's supply of NADH (or other electron donators) and reducing coenzymes (i.e. cytochrome b5) is important for mitigating the formation of MMb and maintaining color stability. Enzymatic and non-enzymatic processes can reduce MMb to DMb which is critical to meat color stability (Bekhit & Faustman, 2005). Reddy and Carpenter (1991) reported that muscles with higher reducing activities also were the muscles that have been traditionally characterized as the most color stable.

The shelf-life of meat is inversely proportional to a muscle's oxygen consumption (Atkins, 1973). If OC rate is low, more oxygen can penetrate inside the muscle and color could be more stable (McKenna et al., 2005). Based on color stability, beef muscles have been categorized as color-stable and color-labile. Muscles that demonstrate greater rates of oxygen consumption (O'Keeffe & Hood, 1982) and lower rates of MetMb reduction (Ledward, 1985) are color-labile. In contrast, muscles with greater reducing activities are the ones that are color-stable (Reddy &

Carpenter, 1981). Muscle tissue characterized by high mitochondrial activity is associated with an increased rate of oxygen consumption and decreased color stability (O'Keeffe & Hood, 1982; Renerre & Labas, 1987; Tang et al., 2005).

2.2 Oxidative changes in meat

Oxidation of meat components has long been identified as one of the primary non-microbial drivers of meat decomposition (Chaijan, 2008; Gray & Monahan, 1992; Renerre, 1990). By definition, oxidation is a chain reaction, three-step mechanism that results in the production of primary oxidation compounds or reactive oxygen species (radicals) which catalyze the oxidation of other meat components (myoglobin, lipids and proteins). The initiation and propagation of oxidation in meats can be fueled by a variety of mechanisms; however, the exposure of meat compounds to endogenous oxidizing chemical species (peroxides, free radicals, etc.), oxidizing environmental components (heat, oxygen, etc.), and catalytic-metal ions are primarily responsible for oxidative deterioration (Martin, 2014).

As stated earlier, the oxidation of the central iron molecule in the heme from the ferrous (Fe^{++} state) to the ferric (Fe^{+++}) state is myoglobin oxidation. It is responsible for discoloration, a change from red OMb to brownish MMb. Muscles that contain greater relative proportions of red fibers, and thus more iron and greater oxygen consumption rates, appear to discolor more quickly (Faustman et al., 2010). Gutzke and Trout (2002) investigated the autoxidation in red-meat Mb from ruminants (beef, lamb, and red deer) and a non-ruminant (pork) and the results indicated that pork Mb less susceptible to autoxidation than its ruminant counterparts.

The oxidation of OMb to MMb generates reactive intermediates capable of enhancing further oxidation of OMb and/or unsaturated fatty acids; specifically, superoxide anion is formed and dismutates rapidly to hydrogen peroxide (H_2O_2). The latter can react with the MMb concurrently generated in this oxidation sequence to form an activated MMb complex capable of enhancing lipid oxidation that is attributed to ferryl-myoglobin. Alkyl hydroperoxides are similar to hydrogen peroxide with respect to their ability to interact with MMb. The MMb-H_2O_2 and ferryl-myoglobin have been documented as potentially powerful contributors to lipid oxidation in meat (Alderton, Faustman, Liebler, & Hill, 2003; Faustman, Liebler, McClure, & Sun, 1999). Lipid oxidation is a free-radical chain mechanism involving initiation, propagation, and termination steps. Substrates necessary for this deteriorative reaction include unsaturated fatty acids, oxygen and chemical species that accelerate oxidation (e.g., iron; Kanner, Shegalovich, Harel, & Hazan, 1988), which are present abundantly in meat displayed aerobically or in high-oxygen modified atmosphere packaging. As initiation occurs oxygen is produced or introduced from the surrounding atmosphere and will attack unsaturated fatty acids, producing more free radicals. This process is known as the propagation step which will continue as long as oxygen is available to cleave the double bonds. Termination will occur naturally as non-reactive products are formed, and the propagation can no longer occur. As detailed by Gray and Monahan (1992), the oxidation of meat lipids results in the formation of primary oxidation (alkyl, alkoxy and peroxy radicals) compounds and reactive oxygen species.

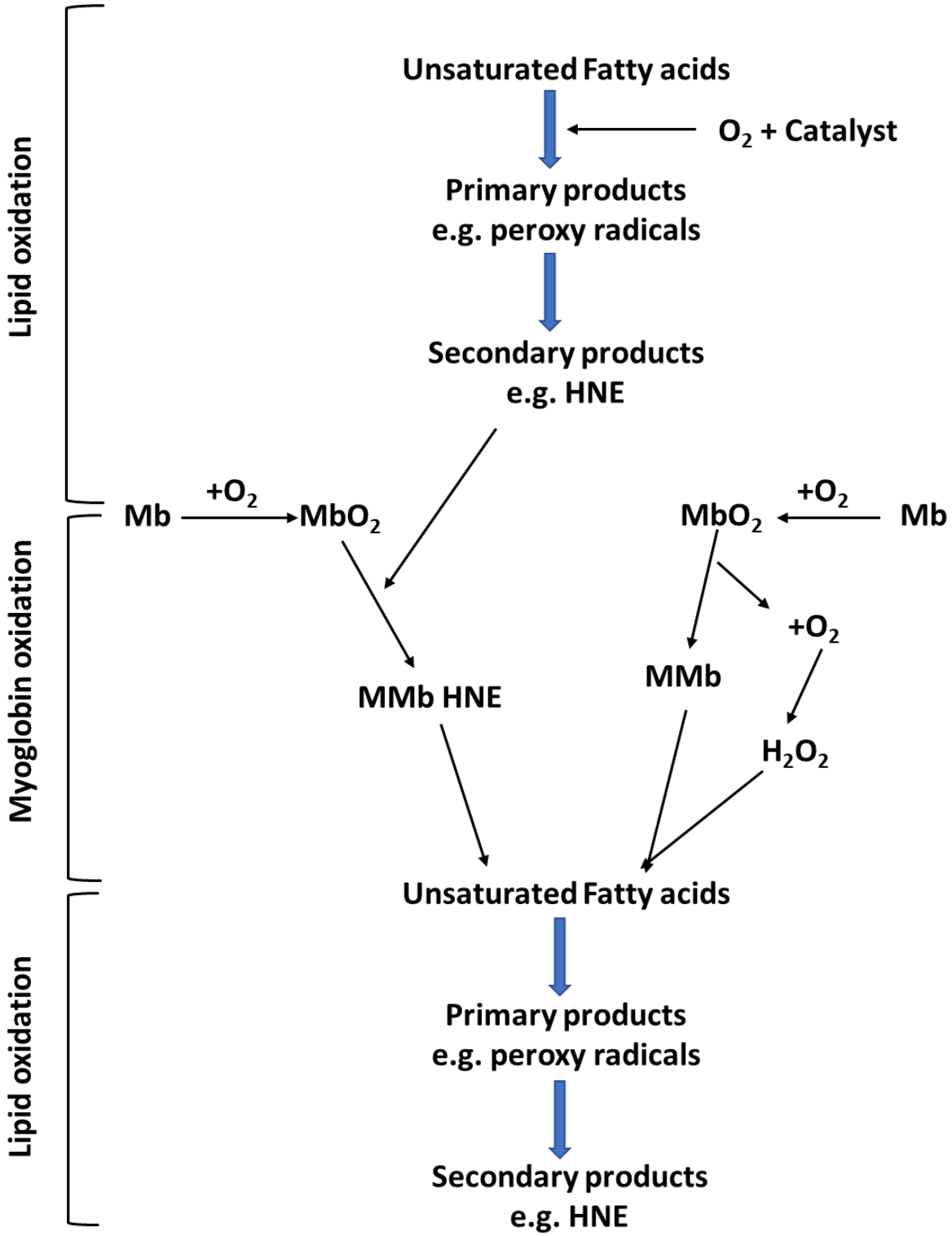
The degree of lipid oxidation in meat is dependent on the composition of the phospholipids, the amount of polyunsaturated fatty acids, and the concentration of pro-oxidants (Calkins & Hodgen, 2007). Although lipid oxidation is usually associated with flavour and odour deterioration, it is also responsible for color changes. The mechanisms by which lipid oxidation could enhance

myoglobin oxidation have been explained primarily on the reactivity of primary and secondary products derived from unsaturated fatty acids (Faustman et al., 2010). Reactive secondary products of lipid oxidation, such as aldehydes, accelerate myoglobin oxidation leading to formation of brown metmyoglobin and subsequent meat discoloration (Faustman et al., 2010).

While generally assumed to be interrelated, the relationship between lipid and pigment oxidation is not well established. It is not thoroughly understood if lipid oxidation precedes pigment oxidation or if pigment oxidation serves as the catalyst for lipid oxidation (Greene et al., 1971). The plausible reason for the strong relationship between pigment and lipid oxidation may be explained by either heme pigments being the catalyst of the propagation step in lipid oxidation or the production of radical species from autoxidation will result in pigment oxidation (Faustman et al., 2010) as described in Figure 2.3.

Several studies have reported that the process of lipid oxidation enhances meat discoloration. Recent investigation of quality parameters in beef packaged under 0%, 10%, 20%, 50% and 80% oxygen (20% CO_2, balance nitrogen) concluded that changes in OMb and a^* values appeared to be driven by lipid oxidation and correlated strongly with TBARS. The mechanisms by which lipid oxidation could enhance myoglobin oxidation have been explained primarily on the reactivity of primary and secondary products derived from unsaturated fatty acids (Zakrys, Hogan, O'sullivan, Allen, & Kerry, 2008). Baron and Andersen (2002) provided compelling evidence that lipid oxidation could enhance myoglobin oxidation based on the reactivity of primary and secondary products derived from unsaturated fatty acids (Zakrys et al., 2008). Baron & Andersen (2002) provided strong evidence for that catalysis of lipid oxidation was due to the abundance of myoglobin and heme iron.

Figure 2.3. Summary of potential interacting oxidation reactions between OMb and unsaturated fatty acids in lipid bilayers. Adapted from Faustman et al. (2010)



Other group of scientists (Alderton et al., 2003; Faustman et al., 1999) demonstrated the *in vitro* oxidation of myoglobin in the presence of 4-hydroxynonenal, a lipid oxidation product. While there exists debate regarding the relationship between pigment and lipid oxidation, the literature suggests undeniable correlations between the two.

2.3. Overview of bison meat

Bison meat became an alternative to beef when Canadian beef consumption per capita decreased abruptly, after the bovine spongiform encephalopathy crisis in 2003 (Steiner et al., 2010). Despite of being rich source of many macro and micro nutrients (Table 2.1.; Canadian Bison Association, 2018) bison meat is not widely available for consumers due to its short shelf life, which limits its expansion.

In the fresh meat marketing, Bison meat colour is consistently unstable (early browning) under retail aerobic packaging conditions (Dhanda et al., 2002; Janz et al., 2000; Pietrasik et al., 2006).

Table 2.1 Nutritional comparison of Bison meat with other meats, per 100 g serving of raw meat (lean steak cuts). Source: Health Canada (2007)

Species	**Total fat (g)**	**Protein (g)**	**Calories (kCal)**	**Cholesterol (mg)**	**Iron (mg)**	**Vitamin B-12 (µg)**
Bison	2.4	23.3	122	65	2.7	1.6
Beef	3.1	22.6	126	52	2.1	2.0
Pork	8.6	22.1	172	54	0.6	0.5
Chicken breast	1.6	22.7	112	58	0.4	0.4
Atlantic salmon	10.9	19.9	183	59	0.4	2.8

The rapid pigment oxidation and surface discolouration mechanisms are not well understood. Bison are more susceptible to the stresses encountered in the pre-slaughter environment (CARC, 2001; loading, transportation, lairage, etc.) which can result in insufficient muscle glycogen degradation and high ultimate pH may occur in bison; however, similar to beef, bison generally reach a normal post-mortem ultimate pH (5.4-5.7; Janz et al., 2001). Structurally, a comparative study characterizing the myoglobin protein between bison and beef determined that bison and beef had the same 100% amino acid sequence and exhibited similar oxidation kinetics and thermostability (Joseph et al., 2010). Consequently, the rapid discolouration in bison meat could not be attributed to biochemistry of bison myoglobin per se. However, bison have been shown to have a difference in muscle fibre type (Koch et al., 1995), resulting in higher pigment concentration (Galbraith et al., 2012) and higher levels of iron (Galbraith et al., 2006; Marchello & Driskell, 2001) compared to those typically found in beef. In addition, polyunsaturated fatty acids (PUFA) in phospholipid membranes are susceptible to oxidative breakdown resulting in changes to the colour (Wood et al., 2008). PUFA levels (weight percentage) in range fed and feedlot fed bison were found to be higher than in range or feedlot fed cattle (Rule, Broughton, Shellito, & Maiorano, 2002). Thus, the relatively rapid deterioration of colour quality of bison muscle may be related to the significantly higher content of both total PUFA and total iron.

The higher PUFA content in bison could be susceptible to oxidation (Pietrasik et al., 2006) by reactive secondary oxidation products such as 4-Hydroxy-2-nonenal (HNE), which accelerates myoglobin oxidation and subsequent meat discoloration (Alderton et al., 2003; Joseph et al., 2010; Lee et al., 2003; Schneider et al., 2001; Suman et al., 2006).

2.4. Protective shelf-life and preservation technologies used in bison meat

A considerable amount of technologies have been applied on bison meat. Most of these post-mortem interventions have proven to increase or stabilize the shelf life and palatability attributes of meat in other species but their application in Bison meat has not been very successful.

One such intervention involves the use of different temperatures employed as a means of cooling bison carcasses in order to avoid meat quality defects including loss of weight, tenderness and inconsistency in colour to name a few. One such study involved the use of elevated temperature conditioning (ETC) in which the internal temperature of *longissimus lumborum* and *semimembranosus* was maintained above 10°C within the first 10 h post mortem. This time/temperature combination resulted in loss of weight, lighter and more intense red colour in ETC samples (than those conventionally chilled) due to accelerated post-mortem glycolysis and pH decline and improved tenderness (Janz et al., 2000). On the other hand, the use of blast chilling (BL) technique in bison by Janz et al. (2001) compromised meat quality resulting in dark meat colour and reduced tenderness measured as increased shear force compared to conventional chilling. However, when BL was done in combination with low voltage electrical stimulation (LVES), the samples from the combined treatment were significantly lighter than conventional chilled samples and improved the tenderness of the meat. Another experiment involved the use of Spray chilling (60-s spray cycles/h for 8 h at 2°C) after which *longissimus* muscles from were removed 24 h post-mortem and stored in vacuum packages for up to 6 weeks, with retail display for 0–9 days. Spray chilling reduced shrink loss but retail display life of steaks was severely limited by early discoloration, an effect exaggerated where the meat was subject to any period of storage (Janz & Aalhus, 2006).

Marination or injection treatments with sodium chloride and sodium tripolyphosphate have also been examined in bison meat. The result of a study by Dhanda et al. (2002) imply that marination reduced shear force of the steaks with no effect on a* and b* values but resulted in darker colour and lowered L* values as compared to control. Pietrasik, Dhanda, Pegg, and Shand (2005) reported lowered tenderness in beef and bison steaks due to other studies also marination/ injection of salt and phosphate.

The combined effects of salt and phosphate injection, modified atmosphere packaging (70% O2 and 30% CO2) and vacuum packaging, storage temperature (–1 °C and +4 °C), and storage time on the color, microbial and oxidative stability of beef and bison *longissimus lumborum* (LL) steaks were investigated. Irrespective of packaging treatments, bison steaks displayed lower OMb levels than beef. Also, Bison steaks developed higher 2-thiobarbituric acid reactive substances (TBARS) during storage (Pietrasik et al., 2006).

Recently a new packaging technology using a vacuum-sealed film containing embedded sodium nitrite has been reported to reduce discoloration in bison meat compared with control PVC overwrapped samples. However, this packaging requires increased time for the meat to brighten up (bloom) and recommends centralized packaging of meat prior to shipment out to retail (López-Campos et al., 2018). These results correspond with another study suggesting improved color stability of Bison meat in nitrite packaging film (Narváez-Bravo et al., 2017).

These technologies have improved, in some cases, organoleptic characteristics and have addressed the issue of stability of bison meat colour to a certain extent but there is still room for more research in this area using different innovative interventions.

2.5. Antioxidant compounds for meat

Meat and meat products are highly susceptible to oxidative deterioration due their rich nutritional composition (Devatkal, Thorat, & Manjunatha, 2014). Antioxidants, intrinsic or added externally, play pivotal role in fighting against these oxidative reactions. In the food industry, these antioxidants can be divided into synthetic and natural antioxidants.

Synthetic antioxidants have been used as antioxidants in various red meat and poultry products (Biswas, Keshri, & Bisht, 2004; Formanek et al., 2001; Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007; McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001); BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate), and TBHQ (tert-butylhydroquinone) are examples of synthetic antioxidants (Balentine, Crandall, O'bryan, Duong, & Pohlman, 2006). However, because of the safety concerns and toxicological effects of these synthetic compounds (De Gonzalez et al., 2008), extensive work is being carried out to find novel and naturally occurring compounds to delay the oxidative degradation of lipids, improve quality, and maintain the nutritional value of foods (De Ciriano et al., 2010; Johnston, Sepe, Miano, Brannan, & Alderton, 2005). Consequently, the food market is demanding natural antioxidants, free of synthetic additives and still orientated to diminish the oxidation processes in high-fat meat and meat products.

Naturally occurring plants are a rich source phenolic compound (De Gonzalez et al., 2008; Kähkönen et al., 1999) and the most important are the tocopherols, flavonoids, and phenolic acids, which can function as antioxidants and provide an alternative to currently used conventional antioxidants. These antioxidants may be found in any plant part, such as grains, fruits, nuts, seeds, leaves, roots, arils, and barks. They prevent the formation of free radicals and

propagation of reactive oxygen species (ROS), whereas other scavenge free radicals and chelate prooxidants (transition metals; Ozsoy, Yilmaz, Kurt, Can, & Yanardag, 2009).

Many natural antioxidants have been studied in meat systems and are reported to be sometimes more active than synthetic antioxidants; the food application of these compounds needs to be explored (Kumar, Yadav, Ahmad, & Narsaiah, 2015). However, the effect natural extracts and essential oils has not been addressed in bison meat, but their effect on beef and other meat products suggest their potential application as an effective antioxidant.

Rosemary and rosemary extracts are some of the most studied natural antioxidants used in meat and poultry products. Ahn, Grun & Fernando (2007) investigated the effects of oleoresin rosemary added at 1% to raw ground beef from which patties were made, cooked, and stored for 9 days at 4 °C and reported reduced TBARS value. However, changes in L*, a* and b* were not positive. Another study included the use of 200 ppm rosemary extract in cooked pork patties and results suggested that rosemary extract significantly reduced lipid oxidation in the patties stored at -25 °C for 10 days (Nissen, Byrne, Bertelsen, & Skibsted, 2004).

As reported by Scramlin et al. (2010), 0.5% oregano oil injections in beef *longissimus dorsi* and *semimembranosus* muscles decreased TBARS values and slowed the rate of decline in a∗ values. Similar results showing reduced oxidation of TBARS in raw and cooked porcine and bovine meat samples (homogenized with 3% oregano w/w) were obtained by Fasseas, Mountzouris, Tarantilis, Polissiou, and Zervas (2008). These observations are also supported by the results obtained by Rojas & Brewer (2008) who studied the effects of water-soluble oregano extract in cooked beef and pork and found that oregano added at 0.02% was effective at reducing lipid oxidation in vacuum-packaged cooked beef samples held at −18 °C for 4 months. They also

reported a reduction in TBARS values with oregano extract added at 0.02% in cooked pork stored under the same conditions, although there was no statistical difference ($P > 0.05$). Additionally, oregano also inhibited lipid oxidation in cooked beef and pork (Rojas & Brewer, 2007) and 85% lean raw beef (Sánchez-Escalante, Djenane, Torrescano, Beltrán, & Roncales, 2003).

CHAPTER III: MATERIALS AND METHOD

This project was reviewed and approved Joint-Faculty Research Ethics Board (JFREB) that operates in accordance with the current Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (Appendix 1), Protocol J2017:105 (HS21275).

3.1. Procurement of samples

A total of ten strip-loins from A1 grade (Canada Gazette, 1992) bison carcasses were obtained from True North Foods, a federally-certified slaughter plant and processing capacity in Carman, Manitoba, Canada. Strip loins were vacuum packaged and shipped to the University of Manitoba Food Science Pilot plant (Winnipeg MB, Canada) within 48 h post-mortem. The strip-loins were kept in their packages and wet aged at 4°C for seven days from the date of slaughter. At the end of the ageing, striploins were unpackaged and subjected to treatments and sampling.

3.2. Application of treatments and sampling

After ageing, a 10 cm thick piece was removed from the strip- loin (posterior end edge) and sub-sampled for oxygen consumption (OC), metmyoglobin reducing activity (MRA). These measurements allowed the establishment of a baseline for the different biochemical components in each subprimal. The remnant muscle was cut into three equal portions, weighed and pH and temperature were measured. Each portion was then randomly assigned to one out of the three treatments i.e. non- enhanced, enhanced with 0.08% oregano extract (Origanox™ WS, Frutarom USA Inc.) and enhanced with 0.05% rosemary (NatureGuard™ - Rosemary extract, Newly

Weds® Foods). The concentration of the extracts in the final product was in accordance to company specification. The water used to make the solutions was distilled and was 5°C when injected. The chemical composition of the extract was determined using GC-MS, and the results are presented in Table 3.1. The non-enhanced strip loins were not injected with water, because previous work has shown that injection of water has little, if any, effect on muscle tenderness (Wheeler, Koohmaraie, & Shackelford, 1997) and because water injection is not used commercially (Diles, Miller, & Owen, 1994). The extracts were injected in the meat using a manual injector pump (Century AC motor, Regal Beloit America, Inc., Tipp City, OH, USA) having 3 spray needles (Presto precision 16-hole spray needles, SNA 4 1/2 H .187, Hantover Inc., Kansas City, MO, USA) at 10% pump level (w/w). The loin portions were injected at all possible points for uniform distribution of treatment in the experimental unit. The injected portions were allowed to equilibrate for 5 minutes and weighed again to ensure retention of 10% extract solution in the muscle. The treated strip loin portions were then sampled for different analysis.

A 2.54 cm steak was cut out of each portion (taking care of no touching the fresh cut surface), weighed (for purge loss calculation), pH determined and placed on a white styrofoam tray having a soaking pad and overwrapped with polyvinyl chloride film (PVC; 037242 PUR Value Polyvinylchloride Standard Meat Films, AGL, Richmond Hill, Ontario, Canada.). Once the trays were prepared, 30 trays (10 strip-loins x 3 treatments) were placed in the coffin-style retail display cabinets (Model M1, Hussmann) at 3 °C under LED lighting (light emitting diodes; Acuity Brands Dimmable Rigid 30-LED Light Strip Board HTG S7 - 94v-0 – 4000k) with intensity 1240 lx. After 1 h exposure to atmospheric oxygen in the retail display cabinet, the steaks bloomed, and colour evaluation was carried out by trained panelists and Minolta

colorimeter. The position of the trays was rotated within the retail display cabinet every 24 hours to ensure similar light and temperature exposure to all steaks as well as to avoid biased evaluation by the trained panel. Both types of colour evaluations, sensory and instrumental, were repeated every 48 h for 5 days (i.e. on d 0, d 2 and d 4) of the retail display period. At the end of the display period and after colour evaluation, the steaks were removed from retail cabinet, weighed and pH was measured. Steaks were cut in half, one half was used for MRA and OC evaluation, the second half was vacuum packaged (6"x10" FlairPak Vacuum Pouch, Flair Flexible Packaging Corporation, Canada/USA) and frozen at -20°C for subsequent lipid oxidation analysis.

Another 2.54 cm steak from each portion was obtained, vacuum packaged and stored at -40 °C for consumer sensory acceptability analysis.

3.3. Extract chemical composition

Extract chemical profiles (volatile and semi-volatile fractions) were assessed using gas chromatography-mass spectrometry (GC-MS). Samples were prepared for analysis by adding 100 μL of extract and 8.5 mL of 1:1 mixture of hexane and acetone in a 20 mL scintillation vial. An aliquot of the mixed solution was then filtered through a 0.45 μm polytetrafluoroethylene syringe filter into an autosampler vial for GC-MS analysis. One microliter of the sample was injected into a GC-MS system (QP2010S, Shimadzu Corp., Kyoto, Japan) with a Phenomenex Z-5HT capillary column. The mass spectral detector was operated from an m/z of 4 to 99. The resulting chromatograms were compared with mass spectral libraries (NIST,Wiley Technology)

to identify unknowns observed. The analysis was conducted by an FDA-registered laboratory, Alliance Technologies (Monmouth Jct., N.J., U.S.A.).

Table 3.1. Comparison of yields of chemical compound obtained for rosemary and extracts using GC-MS.

Tentative Compound ID	Percentage in samples (%)	
	Oregano	**Rosemary**
Benzene, 1,3-bis(1,1-dimethylethyl)-	7.12	2.09
2-Pentanone, 4-hydroxy-4-methyl-	6.05	-
2,4-Dimethyl-1-heptene	4.22	1.01
Palmitic acid	4.15	-
1-Heptanol, 2,4-diethyl-	3.35	-
Heptane, 3,3,5-trimethyl-	2.92	-
Octadecanoic acid	2.69	-
p-Octyloxybenzonitrile	2.65	-
Oleic acid	2.57	-
Tridecanol	2.45	-
1-Decanol, 2-hexyl-	1.66	-
Nonane, 2,6-dimethyl-	1.65	-
2-undecene, 4,5-dimethyl-,	1.51	-
1-Hexadecanol, 3,7,11,15-tetramethyl-	1.35	-
Dodecane, 4,6-dimethyl-	1.29	-

Tentative Compound ID	Percentage in samples (%)	
	Oregano	Rosemary
Phenol, 2,4-bis(1,1-dimethylethyl)-	1.28	-
Oxirane, butyl-	1.28	-
n-Dodecane	1.21	-
Dodecane, 4,6-dimethyl-	1.01	-
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E)-	-	27.44
Heptadecene-(8)-Carbonic Acid-(1)	-	9.32
Stigmast-5-en-3-ol, (3.beta.)-	-	2.78
Isocarnosol	-	2.43
Tetratetracontane	-	2.24
Methyl Commate A	-	2.10
9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	-	1.77
Pentacosane	-	1.53
9-Octadecenoic acid (Z)- (CAS) Oleic acid	-	1.51
Eucalyptol	-	1.25
Norolean-12-Ene	-	1.16
.gamma.-Tocopherol	-	1.12

3.4. pH analysis:

The pH of the treatment solutions (i.e. oregano and rosemary extract solution in water) and the samples was determined using a pH meter with a non-glass probe (HI 99163 Meat pH meter,

Hanna instruments, Carrollton, TX) which was previously calibrated using two buffers (pH 4 and 7). The pH of the treatment solutions and meat samples was measured before applying the solutions to the experimental units. After application of the treatments, muscle pH was measured on the first (d 0) and last day (d 4) of the retail display period. Two measures were taken per steaks by inserting the tip of a pH probe into the steaks on both days and results were averaged prior to statistical analysis.

3.5. Drip loss analysis:

Steaks for color evaluation were weighed (Model R71MHD6, Ohaus Corporation, Parsippany, NJ, USA) prior being overwrapped. At the end of the display period, the steaks were removed from the retail case, re-weighed for steak purge loss calculation. The drip loss is reported as the difference in percentage from the initial weight and final weight and is calculated as:

Drip loss % = [(Initial weight – Final weight) ÷ Initial weight] x 100

3.6. Oxygen consumption:

Oxygen consumption (OC) was determined using the method described in American Meat Science Association meat color measurement guidelines (AMSA, 2012). All samples to be assayed were kept at the same temperature (2 to 4°C) to help ensure uniform oxygenation. A 3 cm × 3 cm × 2 cm cube from each steak sample with minimal visible fat or connective tissue was excised and a thin slice of approximately 2 mm was removed to obtain a minimally handled and pressed fresh cut surface. The freshly cut surface was covered with a smooth layer of oxygen-

permeable polyvinyl chloride film (PVC; 037242 PUR Value Polyvinylchloride Standard Meat Films, AGL, Richmond Hill, Ontario, Canada.) to ensure uniform exposure of the surface to air as well as to avoid drying. After blooming, the PVC film was removed, and the samples were placed in a pouch with very low oxygen permeability (6"x10" FlairPak Vacuum Pouch, Flair Flexible Packaging Corporation, Canada/USA), vacuum packaged using with VAC545, Vac Master with high vacuum and scanned immediately for reflectance from 400 to 700 nm to determine the initial % OMb (Minolta Spectrophotometer, CM-3500d, Minolta Co. Ltd., Japan). The spectrophotometer was calibrated through the vacuum bag film to avoid any artifacts due to the film. Samples were then incubated at room temperature and re-scanning of the same surface was done after 20 minutes. The decline in OMb due to enzyme respiration was measured as an indicator of the tissue's ability to consume oxygen. Oxygen consumption (OC) is reported as the difference in percentage from the first and last measurements and is calculated as:

Oxygen consumption = [(Initial % OMb – Ending % OMb) ÷ Initial % OMb] x 100

3.7. Metmyoglobin reducing activity:

For the measurement of metmyoglobin-reducing activity (MRA), the method described in American Meat Science Association meat color measurement guidelines (AMSA, 2012) was used. A 3 cm × 3 cm × 2 cm cube from each steak sample with minimal visible fat or connective tissue was excised and a thin layer of approximately 2 mm was removed to obtain a minimally handled and pressed fresh cut surface. The surface pigments of the cubed sample were initially oxidized to metmyoglobin (MMb) by submerging the sample in 0.3% sodium nitrite solution for 20 minutes at room temperature which induced MMb formation. After 20 minutes, samples were

removed from $NaNO_2$ solution, blotted dry, placed in a pouch with very low oxygen permeability (6”x10” FlairPak Vacuum Pouch, Flair Flexible Packaging Corporation, Canada/USA), vacuum packaged with VAC545, Vac Master and scanned immediately for reflectance from 400 to 700 nm to determine the initial amount of MMb formed on the surface (Minolta Spectrophotometer, CM-3500d, Minolta Co. Ltd., Japan). The samples were then incubated at 30°C for 2 hours and rescanned to determine the remaining amount of MMb. The reducing ability of the sample was measured as the percentage decrease in surface MMb concentration during the incubation period and was calculated as:

MRA (% of MMb reduced) = [(Initial %MMb − Final %MMb) ÷ Initial %MMb] × 100

3.8. Instrumental color measurement:

Instrumental color was determined a Konica Minolta (Chroma Meter CR-410, Minolta Canada Inc., Mississauga, ON) using illuminant D65 and 10° standard observer angles and 2.54 cm aperture (Commission Internationale de l'eclairage, 1978). The chroma meter was calibrated with the polyvinyl chloride film (PVC; 037242 PUR Value Polyvinylchloride Standard Meat Films, AGL, Richmond Hill, Ontario, Canada.) to avoid any errors in the values. Meat color was measured at three distinct locations across the surface of PVC overwrapped steaks at 1 h post-bloom on day one and every 48 h afterwards for 5 days i.e. on d 0, d 2 and d 4 in the retail display. The CIE L* (lightness), a* (redness) and b* (yellowness) values were measured and hue angle and chroma were calculated as:

Hue angle, HA = [arctangent (b*/a*)]

Chroma, C = [(a*2+b*2)0.5]

3.9. Sensory assessment of color:

A trained sensory panel was used for the sensory assessment of color and discoloration of the treated bison steaks. The panel was screened and trained according to the method described in American Meat Science Association meat color measurement guidelines (AMSA, 2012) based on their ability to discriminate color differences by scoring 50 or less in Farnsworth-Munsell 100-Hue Test. Six panelists who passed the screening test were selected from the department of Food and Human Nutritional Sciences at University of Manitoba and provided training sessions and mock sensory trials. The color evaluation was conducted 1 h post-bloom and every 48 h for 5 days i.e. on d 0, d 2 and d 4 in the retail display. The trained panelists scored the samples through descriptive scale for color from 1 to 8 (1=pale red or pale pinkish red; 2=slightly pale or pale pinkish red; 3= moderately light red or light pinkish red; 4=bright red or pinkish red; 5=slightly dark red or pinkish red; 6= moderately dark red or dark reddish tan; 7=dark red or dark reddish tan or brownish; 8=very dark or tannish red or brown) and for surface discoloration a scale from 1 to 6 (1=no discoloration, 0%; 2=Slight discoloration, 1–20%; 3=small discoloration, 21–40%; 4=modest discoloration, 41–60%; 5=moderate discoloration, 61–80%; 6=extensive discoloration, 81–100%; Appendix 2).

3.10. Lipid oxidation:

Oxidative rancidity of the samples was evaluated by measuring Thiobarbituric Acid Reactive Substances (TBARS) using the modified extraction method described by Buege and Aust (1978). Samples were prepared by blending 10 g of meat sample with 30 ml of distilled water for approximately 60 seconds using a cycle-matic blender (Viking Range, the Middleby

Corporation, USA. The samples were then centrifuged in Sorvall RC 6+ centrifuge (Thermo Scientific) in rotor F13-14x50cy at 1850 x g (3000 rpm) for 10 min at 4°C. From the centrifuged sample, 2 mL of supernatant was transferred into a 15 mL conical centrifuge tube and 4ml TCA/TBA reagent and 100 µL BHA were added to each tube and vortexed thoroughly to incorporate reagents. The sample tubes were heated in boiling water bath (100°C) for 15 min, followed by an ice water bath for 10 min. The tubes were then centrifuged Sorvall RC 6+ centrifuge (Thermo Scientific; rotor F14-6x250y, with 250mL to 15mL conical adapter) at 1850 x g (3000 rpm) for 10 minutes at 4°C. The supernatant was carefully transferred to 1.5 mL semi-micro disposable UV cuvettes and optical density was read using a spectrophotometer (Ultrospec 1100pro, Biochrom Ltd, Cambridge, England) at a wavelength of 531 nm. Samples were analyzed in duplicate (acceptable when intra-sample variation ≤ 10 %) and results were averaged prior to statistical analysis. The TBARS value were expressed as milligram of malonaldialdehyde produced per kilogram of sample and calculated using the prediction equation obtained in standard curve as:

Nmol/g = (OD mean-intercept/slope) x Dilution factor

Mg/kg = (nmol/g x 72)/1000

Note: 72 is a constant (molecular weight of malonaldialdehyde).

3.11. Consumer acceptability test:

For sample preparation, cooking procedures, and cooking equipment, the guidelines described in Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2015) by the American Meat Science Association were

followed. The sample steaks were thawed at 2-4 °C for 24 hours before sensory sessions. The steaks from each extract treatment were assigned and cooked on individual electric grill to avoid contamination of flavour compounds among treatments. Electric grills were preheated (approximately at 165°C) and steaks were placed on them, turned once during cooking (at 35 °C internal temperature) and removed from the grill when they reached the desired internal temperature of 71°C as monitored using meat thermometers. Cooked steaks were trimmed to zero fat cover and 8–15 cubed samples (2.5 cm^3 of size) were excised from each steak and placed in a preheated pan to keep samples warm (at 50ºC) until serving. Temperature of the cooked sampled cubes was monitored with an infrared thermometer (Fisherbrand™ Traceable™ Infrared Thermometer Gun, Fisher Scientific, Canada) to avoid contamination due to handling or thermometer holes in the samples. Cubed samples from each of the three treatments were served warm on pre-coded disposable plastic plates one after the other in a randomized manner. Each panel session lasted approximately 45 minutes.

Consumer sensory panels were conducted over three days in sensory room at the Department of Food and Human Nutritional Sciences, Ellis Building, University of Manitoba, Canada. Ninety panelists were recruited for the test and involved student, staff and professors of the university. All consumers were regular beef eaters. Verbal instructions were provided to each panelist on how to fill out the ballot before serving. The panelists were then seated in individual sensory booth where a ballot containing demographic questionnaire (Appendix 3) and sample evaluation sheets (Appendix 4). Also, disposable plastic cutlery, paper napkin and expectorant cup were provided. Panelists were instructed to cleanse their palate using water, diluted apple juice (10%) and unsalted crackers provided in their booths before tasting the first sample and between samples.

Each panelist was asked to fill out a brief demographic questionnaire before the panel began. Individual panelists were asked to provide the following demographic information: a) gender; b) age; c) ethnic origin; d) household size e) single/dual income household; f) annual household income; g) education level; h) number of times red meat consumed per week including sausages and hamburger.

Consumers were then asked to score the samples through a 9-point hedonic scale (1=dislike extremely; 9=like extremely) for tenderness, juiciness, flavor and overall liking along with the acceptability of all these attributes were asked to identify any kind of off-flavour.

3.12. Statistical analysis:

Data collected were analyzed using PROC MIXED of SAS (Cary, NC) version 9.4 (SAS, 2012). Data for pH was analyzed as a completely randomized design (CRD) with a factorial arrangement. EO treatment (non-enhanced, oregano and rosemary), sampling time (d 0 and 4) and their interactions were considered to be fixed effects. Drip loss, MRA, OC and lipid oxidation data were analyzed as a CRD (EO treatments). Sensory and instrumental colour were analyzed as a CRD with a factorial arrangement, EO treatment, retail display times (d 0, 2 and 4) and their interactions were considered to be fixed effects (using day as a repeated measure with autoregressive [AR] covariance structure). In this group of data, loin side within treatment was incorporated into the model as a random effect.

Palatability attribute data was analyzed as a randomized block design. Thus, the model contained extract treatment as the main effect, and the consumer panelist were considered as a random

variable (block). Chi-squared analysis was performed for demographic data, overall acceptability, and flavour profile.

CHAPTER IV: RESULTS AND DISCUSSIONS

4.1 pH:

The pH of the rosemary and oregano injection solutions were 6.8 and 4.45, respectively. However, the injection solutions did not cause any pH changes in the treated bison meat (P = 0.73; Table 4.1.). Regardless of the injection enhancement treatment, the pH in all steaks decreased over time, being more acidic at d 4 of the retail display period ($P < 0.05$; d 0 = 5.76 vs d 4 = 5.63).

Table 4.1 Changes on physical and metabolic parameters of bison steak injected with oregano and rosemary extracts during retail display period.

Variable	Reference[1]	Control	Oregano	Rosemary	SEM
pH	-	5.71	5.72	5.68	0.03
Drip loss, %	-	3.54[a]	7.07[b]	6.47[b]	0.34
OC	31.07[ab]	44.52[a]	24.06[b]	35.64[a]	6.91
MRA	51.88[b]	42.62[a]	49.31[b]	40.46[a]	3.69

[ab] Means with different superscripts were significantly different ($P < 0.05$).

SEM: standard error of the mean.

[1]Reference: steak obtained at d 0 before cutting the striploin into three equal portions and allotted to 1 out of 3 treatments with essential oils.

OC: oxygen consumption

MRA: Metmyoglobin reducing activity

In agreement with the current study, Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores & Legarreta (2009) indicated that pH changes occurred as a function of time and not due to plant extract incorporation. The author found a decrease in pH because storage period alone (after 72 hours) rather than due to applied oregano and rosemary extracts (hundred grams mixed with 0.02% w/w of extract/lard) in pork batter. These results are in accordance with Balentine et al. (2006) who speculated that the reduction in pH might be attributed to the production of acids by meat spoilage bacteria. On the other hand, Sánchez-Escalante et al. (2003) indicated no significant differences either among herb extract treatments or the storage time in beef patties treated with oregano (0.01 and 0.02%) and rosemary (0.1%) extracts in modified atmosphere packaging. The findings from another research showed that injecting oregano solution (0.5% oregano oil solution injected at 10% level w/w) had no differences on the pH of beef *longissimus dorsi* (Scramlin et al., 2010).

4.2 Drip loss analysis:

Drip loss percentage of all the samples in retail display was analysed at the end of display period Least square means for drip loss for all three treatments are presented in Table 4.1. Oregano and rosemary injected steaks resulted in more purge loss than the control steaks ($P > 0.05$) at the end of display period (d 4).

Publications addressing the effect of injecting herb extract solutions on purge/drip loss do not exist to author's knowledge. However, even in fresh meat, a certain loss of water is inevitable. Therefore, in this case the extra loss of fluid in treated samples may be due to the extraneous injection of solution in the samples.

4.3 Oxygen consumption:

Table 4.1. depicts the effects of herb extract treatments on oxygen consumption of the bison steaks. No significant difference was detected between oregano treated steaks (d 4) and reference steaks (d 0) in OC values. However, oregano treatment had significantly lower OC values with respect to control and rosemary treated steaks ($P < 0.05$). On the other hand, rosemary steaks did not show statistical difference in comparison to control ($P > 0.05$).

The application of oregano extract has the potential to reduce or maintain the OC in fresh bison meat for 5 days. Reduced OC is favourable for increased meat color stability. This fact is supported by an experiment by Atkinson and Follett, (1973), who reported that lower levels of oxygen uptake by mitochondria and/or sub-mitochondrial particles in beef muscle showed longest display life, while lamb muscle having greatest oxygen uptake displayed shortest display life.

Also, several studies in literature suggest that increased OC decreases color stability because less oxygen is available to bind with Mb, creating oxidative conditions that favor metmyoglobin formation (Ledward, 1985; O'Keeffe & Hood, 1982; McKenna et al., 2005). However, data supporting the role of antioxidant extracts on OC of bison meat does not exist in literature.

4.4. Metmyoglobin reducing activity.

The least square mean values for MRA due to the effects of treatments on the bison steaks are shown in table 4.1. The MRA values from oregano steaks were similar to those reference steaks ($P > 0.05$). In contrast, MRA values declined considerably in control and rosemary in

comparison to oregano and reference steaks ($P < 0.05$). No significant difference was detected between rosemary and control steaks ($P > 0.05$)

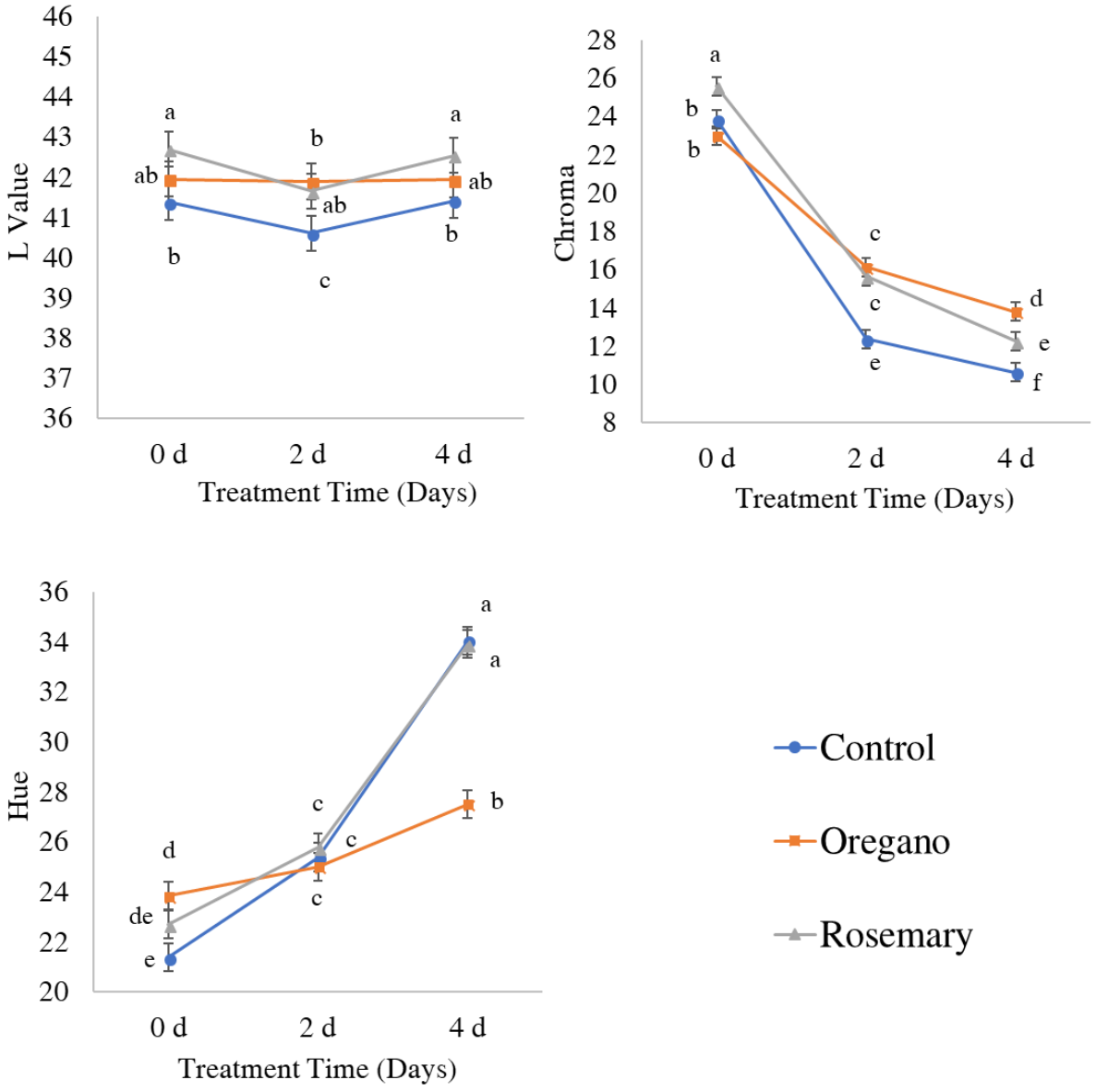
These results indicate that application of oregano extract promotes improved metmyoglobin reducing activity although the mechanisms by which oregano extract can maintain or enhance meat MRA values are not well understood. Metmyoglobin reducing systems (enzymatic and non-enzymatic) give meat the ability to maintain the heme iron in a reduced state to preserve desirable levels of oxymyoglobin (Bekhit & Faustman, 2005). However, it is unlikely that the addition of extracts (antioxidants) are replenishing the reducing pool. Instead, it is more plausible that the addition of antioxidants is alleviating the rapid utilization of oxidative enzymes which contribute to colour stability (Martin, 2014).

The MRA is an important inherent property that extends colour life. Nonetheless, the studies that determined the effects of antioxidants on MRA are still limited. One such study was conducted (English et al., 2015) on the effects of water- and oil-based rosemary (0.2% w/w) on ground beef in retail as well as high oxygen modified atmosphere packaging systems. The results from this study indicated that rosemary treatments increased MRA and presented stable colour in both packaging systems. This study does not agree with our results. The reason behind the disagreement of results in this study and our results may be because of the use of different percentage of extract or the extracts used might have had different antioxidant potential and composition.

4.5. Instrumental colour measurement:

Least-squares means for CIE L*, hue and chroma values of the steaks across the 5 days of simulated retail display are presented in Figure 4.1. Objectives traits were affected by treatment x display time interaction ($P < 0.05$).

Figure 4.1. Changes in CIE L* (SEM = 0.44), hue (SEM = 0.56) and chroma (SEM = 0.48) measurements during retail display (0d to 4d) for control, oregano and rosemary treated steaks.



Except for d 2, treatments presented similar L* values at the beginning and the end of the retail display period. Rosemary steaks had higher L* values than control steaks in each day ($P < 0.05$) while oregano steaks had intermediate values ($P > 0.05$). The Chroma value decreased for all three treatments (less vivid red colour) as the days of retail display increased, but the strip-loin steaks injected with oregano had a lesser pronounced decline of Chroma values than the other two treatments ($P < 0.05$). The Hue values increased for all three treatments as the display days increased, but the increase of hue in oregano samples was much less than the other two treatments such that it had a lower Hue values at the end of the retail display (lower value indicates smaller changes in red colour).

In contrast, results reported by Hernández-Hernández et al. (2009) suggest that herb extracts did not affect chroma values in pork when was incorporated rosemary and oregano extracts (0.02% w/w) were incorporated in combination with ethanol and/or chloroform. Several studies using oregano and rosemary extracts in beef (loin and patties) and pork (Balentine et al., 2006; Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011; Sánchez-Escalante, Djenane, Torrescano, Beltrán, & Roncalés, 2001; Sánchez-Escalante et al., 2003) also reported higher a* values in oregano or rosemary treated meat than control. In the current study, hue (0° = red through 90° = yellow, 180° = green, 270° = blue and back to 0°) and chroma (0 = completely unsaturated i.e. a neutral grey, black or white to 100 = very high saturation or colour purity) values indicate more stable red colour in oregano injected steaks.

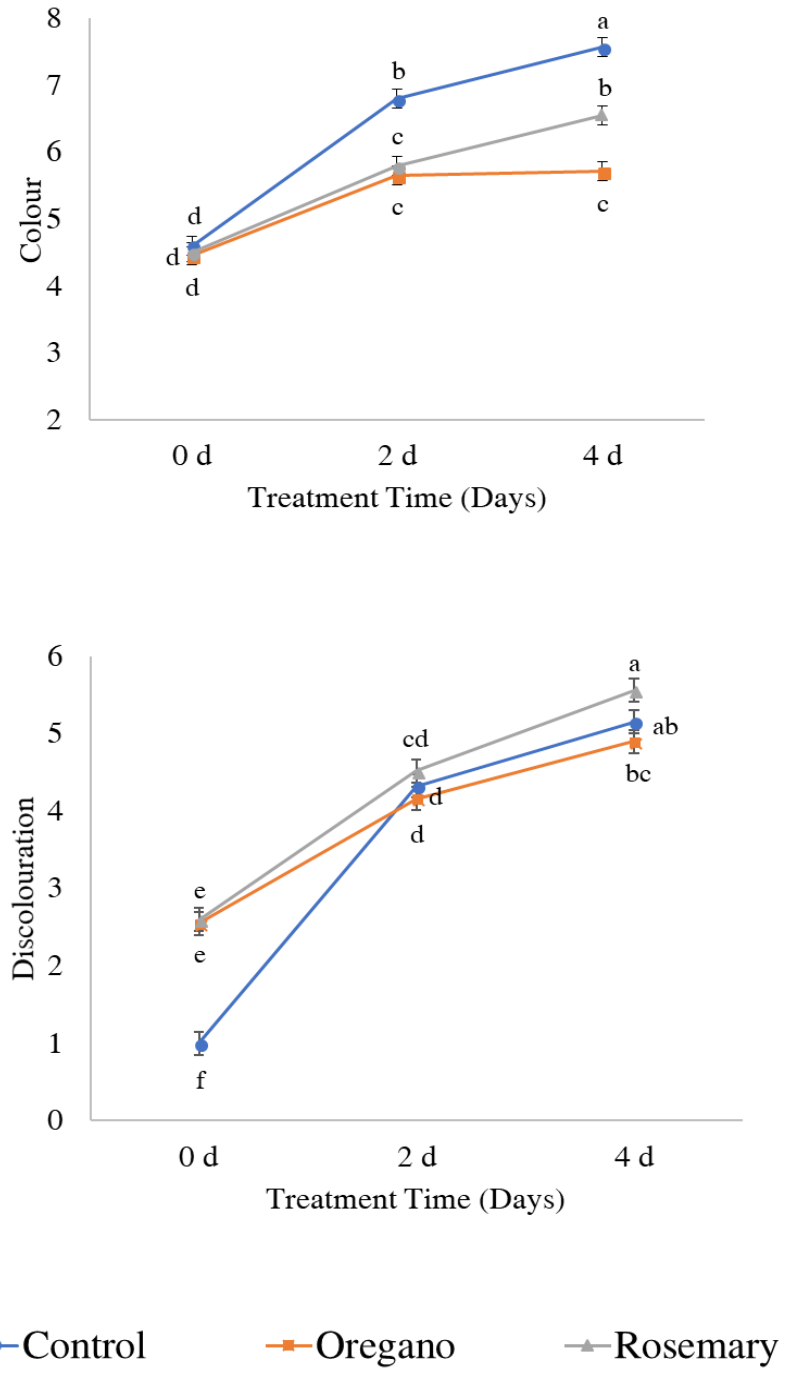
4.6. Sensory assessment of colour:

The analysis of variance detected significant difference in colour and discolouration scores due to treatment × retail display time ($P < 0.05$). The average score of trained panelists for lean colour and surface discolouration are presented in Figs. 4.2.

The panelist scores indicated that lean colour did not differ between treatments upon initiation of display ("bright cherry red"; $P > 0.05$), however, oregano and rosemary steaks scored higher for surface discolouration (slight to small discolouration, 0-40%) than control (no discolouration). This could possibly be due to the use of manual injectors for injecting treatment solution in the meat and artifacts due to needle puncturing might have appeared as discoloured meat surfaces to the panelists. At day 3, herb extract treatment steaks showed lower color display scores than the control samples ($P < 0.05$), but discolouration score did not differ between treatments at day 3 ($P > 0.05$; modest discolouration, 40-60%). At the end of the retail display (d 4), oregano steaks showed a lower colour score ("slight dark red") followed by rosemary ("moderately dark red to brownish colour") and then control ("very dark or brown colour") ($P < 0.05$). On the other hand, oregano steaks exhibited lower discolouration scores (moderate discoloration, 60-80%) than rosemary steaks (Extensive discoloration, 80-100%; $P > 0.05$); control steaks had intermediate values. This implies that oregano steaks presented a more stable red colour display than rosemary and control steaks.

Camo et al. (2011) studied the effect of different concentrations of oregano ranging from 0.5% to 4% in the form of edible coating and spray. The author found that any oregano concentration in both application forms reduced the darkening of red colour of meat and well as surface discolouration after 14 d of storage.

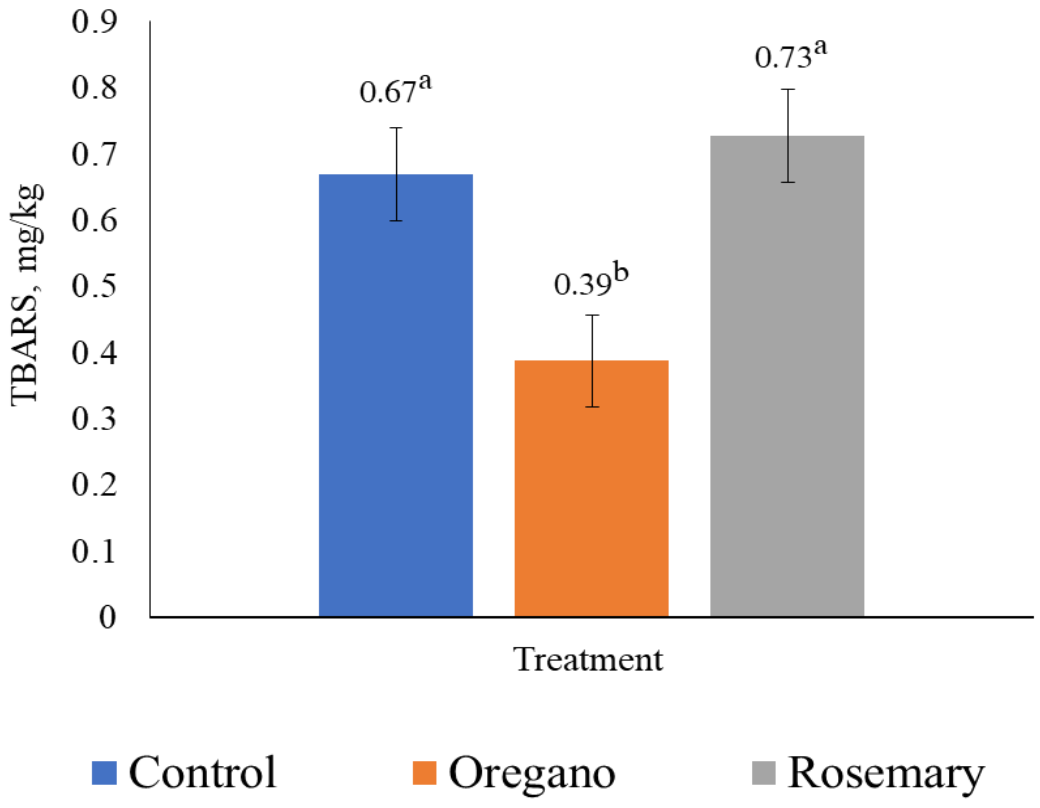
Figure 4.2. Trained panelists colour (1=pale red or pale pinkish red to 8=very dark or tannish red or brown; SEM = 0.14) and surface discolouration scores (1 = 0% to 6 = 100%; SEM = 0.15) for control, oregano and rosemary treated steaks over 5 days of retail display period.



4.7. Lipid oxidation:

The values of thiobarbituric acid reactive substances, representative of lipid oxidation, are shown in Figure 4.3. Oregano extract samples had lower TBARS values compared to control and rosemary steaks ($P < 0.05$), indicating high protection of meat against lipid oxidation. Contrarily, rosemary extract increased the lipid oxidation of meat reaching the same level of TBARS values as the control ($P > 0.05$).

Figure 4.3. TBARS in strip-loin steaks for control, oregano, and rosemary treatments at the end of retail display period. SEM = 0.07.



The results from our study correspond to the results presented by Scramlin et al., (2010) involving incorporation of 0.5% oregano oil solution (injected at 10% level w/w) in intact beef muscles *longissimus dorsi* and *semimembranosus* where oregano treatment caused a reduction in oxidation of lipids in oregano compared to the control samples ($P < 0.05$).

Oregano and rosemary extracts presented dissimilar effect on TBARS values and this may be due to their differences in antioxidant constituents. The GC-MS analysis of oregano extract found 9.75% of antioxidants were composed with phenylpropanes (7.12%; Benzene, 1,3-bis[1,1-dimethylethyl]-), phenolic compounds (1.28% Phenol, 2,4-bis[1,1-dimethylethyl]-) and fatty alcohols (1.35%; 1-Hexadecanol, 3,7,11,15-tetramethyl-); while rosemary presented 9.67% of antioxidants as aromatic hydrocarbon (2.09%; Benzene, 1,3-bis[1,1-dimethylethyl]-), sterol (2.78%; Stigmast-5-en-3-ol, [3.beta.]-), phenolic diterpene (2.43%; Isocarnosol), cyclic ether & monoterpenoid (1.25%; Eucalyptol), and methylated phenol (1.12%; gamma-Tocopherol). Some of these compounds have been reported to have strong antioxidant properties such as phenolic and terpenoid components (Bakkali, Averbeck, Averbeck & Idaomar, 2008; Raman et al., 2012) and both extracts presented similar amount of these compounds; nevertheless, phenylpropanes compound was a major antioxidant constituent in oregano extract, which has shown antioxidant properties *in-vitro* (López, Jäger, Akerreta, Cavero & Calvo, 2010). It is noteworthy, even though rosemary extract presented phenolic and terpenoid components, the extract did not show certain level of antioxidant effect in the experiment.

Differences may also be attributed to the different concentrations of the extracts used. This assumption is supported by a study on beef patties containing oregano (0.1 and 0.02%) and rosemary (0.1%) extracts and stored for 16 days in high oxygen modified atmosphere packaging (Sánchez-Escalante et al., 2003). When oregano was used at a higher concentration (0.1%), the

antioxidant effect was significantly higher than treatment with 0.02% oregano; while the same concentrations of rosemary and oregano (0.1%) exhibited similar effects. Additionally, regardless extract concentration, all the extracts reduced TBARS formation compared to the control group. However, rosemary concentration applied in the current study (0.05%) did not mitigate the lipid oxidation.

The potential of antioxidant application of oregano and rosemary towards the mitigation of lipid oxidation in meat and meat products has been well documented (Camo, Beltrán, & Roncalés, 2008; Camo et al., 2011; Jayasena & Jo, 2014). Specifically, the inhibition of oxidation may be attributed to the termination of free radical chain-reaction and the sequestering of oxidative species in stored meat.

4.8. Consumer acceptability test:

Demographic information was obtained from consumer panelists (N = 90; Appendix 1) and analysed using frequency analysis. Sensory analysis in the current study was performed by a mixed sample population of male (46%) and female (54%) consumers. Panelists between 18 to 34 years of age accounted for the greatest percent (68%) of the sample population. All the study participants were regular beef eaters (98% consume once or more than once a week) with various level of education (69% had college or post-graduate level), annual income (62% had less than $ 69,999), and ethnicity (39% white followed by 28% Chinese, 7% both South Asians and South-east Asians, 6% black, 4% aboriginal people as well as Latin Americans, 3% West Asians and 2% others; Appendix 5).

The results from consumer analysis are presented in Table 4.2. The analysis of variance detected that overall liking, tenderness and juiciness were affected by extract treatments where oregano and rosemary extract treated steaks were not significantly different to the control group ($P > 0.05$). On the other hand, a tendency was observed on flavour score ($P = 0.06$), where consumers provided a higher score to rosemary and control than oregano. Also, when the percentages of acceptability were compared, a tendency was observed ($P = 0.06$), where the highest percentage of overall acceptability was obtained by steaks treated with rosemary (86.52%), followed by control (76.40%) and oregano (72.22%). The results from flavour testing exhibited that all treatments had the same flavour profile ($P = 0.14$; Figure 4.4), with the most common flavour descriptors being: unidentified (29.10%), serumy/bloody (15.30%), livery (11.57%) and grainy (10.82%) flavours.

Table 4.2. Consumer panel results for sensory attributes of treated bison steaks for overall liking, flavor, tenderness, juiciness and acceptability %

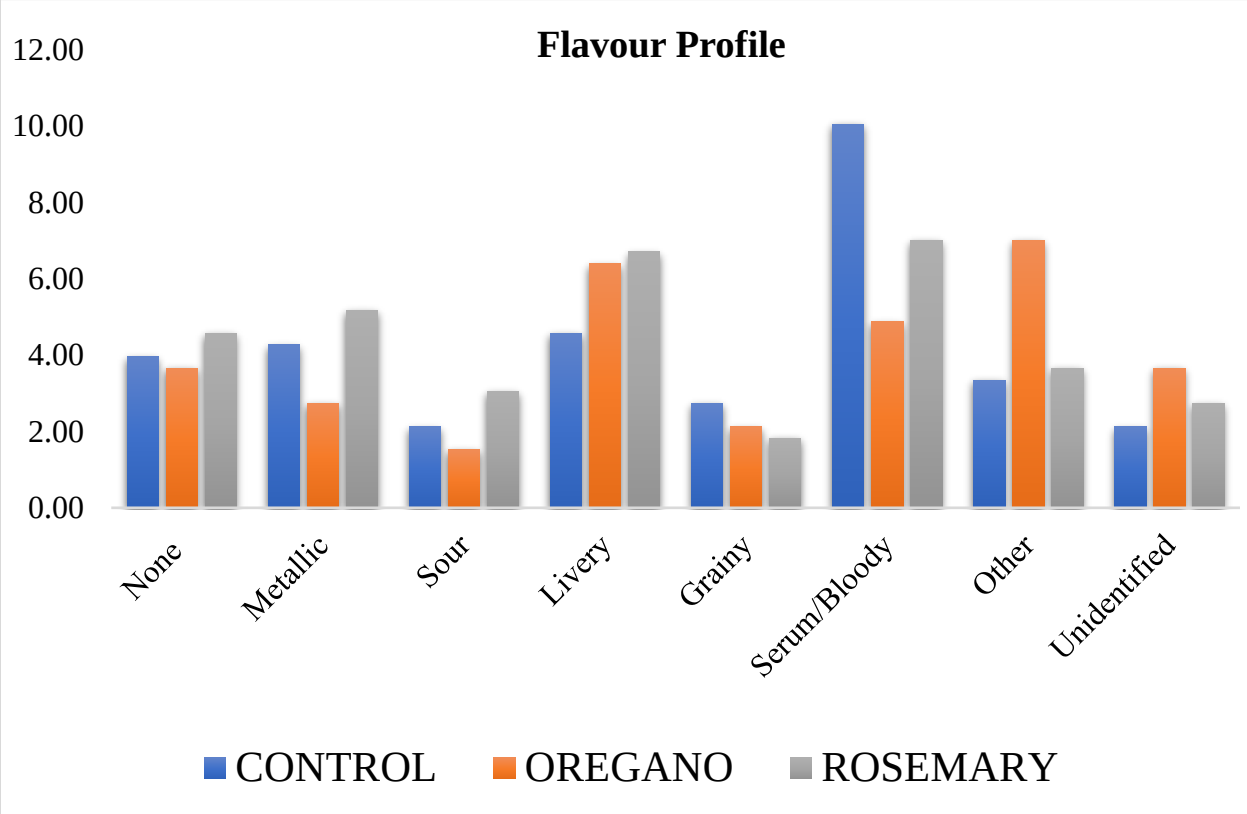
Variable	Control	Oregano	Rosemary	SEM	P-value
Overall liking[a]	6.28[ab]	6.20[a]	6.58[b]	0.17	0.02
Flavour	6.20	5.84	6.39	0.20	0.06
Tenderness	6.48[ab]	6.04[a]	6.86[b]	0.19	< 0.01
Juiciness[a]	6.49[ab]	6.12[a]	6.64[b]	0.17	0.04
Acceptability (%)[B]	76.40	72.22	86.52	-	0.06

[A]Hedonic-point scale where 1 = Dislike extremely, and 9 = Like extremely.

[B]Consumers were asked to indicate if overall liking was acceptable or unacceptable.

Therefore, rosemary injected steaks were more desirable and palatable than oregano counterpart. Nevertheless, all treatments had the same score range, which corresponded to a score of 6, “Like slightly” of a 9-point hedonic scale used in the experiment.

Figure 4.4. Flavour profile of control, oregano and rosemary treated steaks judged by a consumer panel (n = 90). The frequency distribution was tested for significance using a chi-square test (P = 0.14) and the values are expressed in percentage.



The results of the present study tend to be somewhat opposite to those obtained by Scramlin et al. (2010) who studied the effect of oregano oil (0.5%) on palatability variations in beef *longissimus* and found no effect on tenderness and juiciness attributes compared to control but unacceptable scores for off-flavor and overall acceptability. Camo et al. (2011) applied different

concentrations of oregano ranging from 0.5% to 4% in form of an edible coating and spray, and found that in both forms, the higher concentration treatment (4%) had unacceptable strong oregano odour. In contrast, Al-Hijazeen, Lee, Mendonca, and Ahn (2016) applied oregano EO (0 to 200mg/kg) plus tannic acid (5 to 10 ppm) in ground chicken; and concluded that regardless of tannic acid concentration, ground chicken treated with 200 mg/kg of oregano EOs had greater overall acceptability ($P < 0.05$) than control. In the case of other EOs, Mohamed and Mansour (2012) added marjoram and rosemary EOs at 200 mg/kg to beef patties formulated with 200 g/kg mechanically deboned poultry meat and reported that both EOs increased the overall acceptability of the patties ($P < 0.05$) with respect to the control group.

CONCLUSIONS

The injection of oregano plant extracts provides colour stability during retail display (more red and vivid colour) based on their antioxidant activity, and its potential to increase the MRA capacity in the bison meat. Additionally, the injections of both plants extract solutions, oregano and rosemary, do not confer negative changes to the palatability attributes and flavour profile. However, rosemary treated bison steaks were appreciated by the consumers as more palatable.

Based on these results, plant extracts were able to activate enzyme or protein groups (such as reducing enzymes, oxygen scavenging enzymes, and mitochondria) related to the colour stability, so their action mechanisms could be beyond of H-donating activity or high radical-absorbance capacity. The ability to increase MRA capacity in the bison meat could be related to activation of sarcoplasmic proteins, which play a critical role in fresh meat colour due to their ability to interact directly with myoglobin. Changes in the balance between antioxidant/pro-oxidant components in the sarcoplasm can induce lipid oxidation and cause meat discolouration. In consequence, plant extract could stimulate this group of proteins regenerating NADH and scavenge superoxide anions for subsequent metmyoglobin reduction. Nonetheless, no studies are available that have examined the differential abundance of several proteins, of bison aged muscles enhanced with plant extracts and need to be evaluated.

Also, further research needs to be conducted if plant extracts can reduce protein oxidation in bison meat and how the mechanisms behind the interrelationships between lipid and protein oxidation work.

APPENDICES

Appendix 1: Ethics Approval

PANEL ON RESEARCH ETHICS
Navigating the ethics of human research

TCPS 2: CORE

Certificate of Completion

This document certifies that

Vipasha Sood

has completed the Tri-Council Policy Statement:
Ethical Conduct for Research Involving Humans
Course on Research Ethics (TCPS 2: CORE)

Date of Issue: **22 August, 2017**

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PROTOCOL APPROVAL

TO: **Vipasha Sood** **(Advisor: Argenis Rodas-González)**
Principal Investigator

FROM: **Kevin Russell, Chair**
Joint-Faculty Research Ethics Board (JFREB)

[redacted]

Re: **Protocol J2017:105 (HS21275)**
"Improving shelf life of fresh bison steaks treated with oregano and rosemary essential oils"

Effective: December 21 2017 **Expiry:** December 21, 2018

Joint-Faculty Research Ethics Board (JFREB) has reviewed and approved the above research. JFREB is constituted and operates in accordance with the current *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans.*

This approval is subject to the following conditions:

1. Approval is granted only for the research and purposes described in the application.
2. Any modification to the research must be submitted to JFREB for approval before implementation.
3. Any deviations to the research or adverse events must be submitted to JFREB as soon as possible.
4. This approval is valid for one year only and a Renewal Request must be submitted and approved by the above expiry date.
5. A Study Closure form must be submitted to JFREB when the research is complete or terminated.
6. The University of Manitoba may request to review research documentation from this project to demonstrate compliance with this approved protocol and the University of Manitoba *Ethics of Research Involving Humans.*

Funded Protocols:
- **Please mail/e-mail a copy of this Approval, identifying the related UM Project Number, to the Research Grants Officer in ORS.**

Appendix 2: Trained sensory color evaluation form

Trained Color Evaluation

Project: Bison steaks with antioxidant extracts

NAME: _____ DATE _____

Muscle Color Score Scale	**Discoloration Score Scale**
1. Pale red or pale pinkish red	**Surface % MMb**
2. Slight Pale or pale pinkish red	1. None (0%)
3. Moderately light red or light pinkish red	2. Slight discoloration (1-20%)
4. Bright red or pinkish red	3. Small discoloration (21-40%)
5. Slightly dark red or pinkish red	4. Modest discoloration (41-60%)
6. Moderately dark red or dark reddish tan	5. Moderate discoloration (61-80%)
7. Dark red or dark reddish tan or brownish	6. Extensive discoloration (81-100%)
8. Very dark red or tannish red or brown	****Score to whole point increments only****
****Score to half-point (0.5) increments****	

No.	**Color**	**Surface**		**No.**	**Color**	**Surface**	**No.**	**Color**	**Surface**	**No.**	**Color**	**Surface**
1				21			41			61		
2				22			42			62		
3				23			43			63		
4				24			44			64		
5				25			45			65		
6				26			46			66		
7				27			47			67		
8				28			48			68		
9				29			49			69		
10				30			50			70		
11				31			51			71		
12				32			52			72		
13				33			53			73		
14				34			54			74		
15				35			55			75		
16				36			56			76		
17				37			57			77		
18				38			58			78		
19				39			59			79		
20				40			60			80		

Appendix 3: Demographic questionnaire used for consumer palatability and acceptability testing.

Date: _____ Panel Number: _____ Session: _____

Thank you for your participation today with our meat tasting.

Before you begin, please **listen** to the instructions on how to use the scales contained in this questionnaire.

In between each sample, please **cleanse** your palate by first taking a sip of diluted Apple Juice then chewing a piece of cracker and then taking another sip of diluted Apple Juice. We have also supplied water to cleanse your palate.

We are after your opinion, and therefore ask that you **do not talk** to anyone else in the room during the research session.

Now just a few questions about yourself, please check the appropriate box. (All this information is strictly confidential)

Demographic data
(Please check the one answer that applies for each item.)

What is your gender? □ Male □ Female

What is your age? □ 18-34 □ 35-50 □ Over 50

What is your ethnic origin?

□ Aboriginal person (First Nations, Métis or Inuk)
□ South Asian (e.g., East Indian, Pakistani, Sri Lankan, etc.)
□ Southeast Asian (e.g., Vietnamese, Cambodian, Laotian, Thai, etc.)
□ Latin American
□ West Asian (e.g., Iranian, Afghan, etc.)
□ Filipino
□ Other - specify:
□ White
□ Chinese
□ Japanese
□ Black
□ Arab
□ Korean

What is the number of people in your home? 88

□ 1 □ 2 □ 3 □ 4

□ 5 □ 6 □ More than 6

Is your household a single or dual income household?

□ Single Income □ Dual Income

Annual Household Income (if you are a full-time student indicate your parents' income)

□ Under $20,000 □ $50,000 - $69,999

□ $20,000 - $29,999 □ $70,000 - $89,999

□ $30,000 - $49,999 □ $90,000 or more

Education level

□ Non-High School graduate □ College Graduate

□ High School graduate □ Post-Graduate

□ Some college/Technical School

How many times a week do you consume red meat? Including beef sausage and hamburger

□ None □ 1-2 □ 3-4

□ 5-6 7 □ or more

Thank you very much for participating

Appendix 4: Consumer evaluation form for organoleptic sensory test.

Consumer Evaluation Form for SAMPLE #:_____

Instructions: Thank you for your participation in our taste test. You will be served three government inspected and approved bison steaks to taste. Take your time and don't hesitate to ask any questions. Thanks again!

Please indicate how much you ***like or dislike*** the sample by placing a check in the appropriate shaded box.

	Tenderness	Juiciness	Flavor	Overall Like
9. Like Extremely				
8. Like Very Much				
7. Like Moderately				
6. Like Slightly				
5. Neither Like or Dislike				
4. Dislike Slightly				
3. Dislike Moderately				
2. Dislike very much				
1. Dislike extremely				
	Was TENDERNESS acceptable Yes_____ No_____	**Was JUICINESS acceptable** Yes_____ No_____	**Was FLAVOR acceptable** Yes_____ No_____ **What kind of flavour did you detect?** None __ Metallic __ Sour __ Livery __ Grainy __ Serum/Bloody __ Other __ Unidentified __	**Was the sample acceptable OVERALL** Yes_____ No_____

Appendix 5: Demographic characteristics of consumer sensory study participants (N = 90)

Characteristic	Response	Percentage of participants
Gender	Male	45.56
	Female	54.44
Age	18-34	67.78
	35-50	23.33
	Over 50	8.89
Number of times beef is consumed per week	None	2.22
	1-2	36.67
	3-4	42.22
	5-6	17.78
	7 or more	1.11
Household size	1 person	10
	2 persons	24.44
	3 persons	22.22
	4 persons	20.00
	5 persons	10.00
	6 persons	8.89
	More than 7 persons	4.44
Ethnic Origin	Aboriginal person (First Nations, Métis or Inuk)	4.44
	White	38.89
	South Asian (East Indian, Pakistani, Sri Lankan, etc.)	6.67
	Chinese	27.78
	Southeast Asian (Vietnamese, Cambodian, Thai, etc.)	6.67
	Japanese	0
	Latin American	4.44
	Black	5.56
	West Asian (e.g., Iranian, Afghan, etc.)	3.33
	Arab	0
	Filipino	0
	Korean	0
	Other	2.22
Single or Dual Income Household	Single Income	43.33
	Dual Income	56.67

Annual	Under $20,000	11.11
Household	$20,000 - $29,999	20.00
Income	$30,000 - $49,999	10.00
	$50,000 - $69,999	21.11
	$70,000 - $89,999	17.78
	$90,000 or more	20.00
Education level	Non-High School graduate	0
	High School graduate	17.78
	Some college/Technical School	13.33
	College Graduate	21.11
	Post-Graduate	47.78

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