FORMATION OF KEY FLAVOUR PRECURSORS IN BISON *LONGISSIMUS DORSI* MUSCLE: EFFECT OF CHILLED STORAGE CONDITIONING

by

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Abstract

Water and lipid-soluble meat flavour precursors are gradually formed *post-mortem* via biochemical reactions. Storage time and temperature can affect final flavour precursor concentrations which in turn will affect the sensory quality of cooked meat. Selected key flavour precursors were monitored in *Bison bison longissimus dorsi* muscles from six animals stored at 2, 4, 8, 15 and 21 days at 4°C, in order to evaluate the effect of *post-mortem* conditioning on the formation of flavour precursors. Results were correlated with sensory data obtained using quantitative descriptive analysis with 8 trained panelists. While lipid-soluble flavour precursors remained mostly unchanged, significant increases ($P<0.05$) in concentrations of water-soluble flavour precursors including reducing sugars (eg. ribose, xylose), free amino acids (eg. valine, leucine) and adenosine-5'-triphosphate (ATP) degradation products (eg. inosine and hypoxanthine) were obtained with chilled storage conditioning *post-mortem*. The overall balance and correlations of water-soluble flavour precursors with storage day 15 and 21 were reported and can potentially impact the eating quality of cooked bison meat.

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List of Abbreviations

AMP	adenosine 5’-monophosphate
ANOVA	analysis of variance
ANSA	8’-anilino-1-naphthalene sulfonic acid
ATP	adenosine 5’-triphosphate
BF	*biceps femoris*
BSTFA	N,O-bis(trimethylsilyl) trifluoroacetamide
C:M	chloroform:methanol
$CaCl_2$	calcium chloride
CE	cholesterol ester
CFIA	Canadian Food Inspection Agency
DG	diglyceride
EI	electron impact
F6P	fructose 6-phosphate
FFA	free fatty acid
FHE	Faculty of Human Ecology
G6P	glucose 6-phosphate
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GG	*gastrocgnemius*
GM	*gluteus medius*
GMP	guanosine 5’-monophosphate
HPLC	high pressure liquid chromatography
IMP	inosine 5’-monophosphate
IS	internal standard
KH_2PO_4	potassium phosphate
LD	*longissimus dorsi*

LL	*longissimus lumboram*
LT	*longissimus thoracis*
M6P	mannose 6-phosphate
MONO	monounsaturated fatty acid
PCA	Principal Component Analysis
PL	phospholipids
PLS	partial least squares
PM	psoas major
PUFA	polyunsaturated fatty acid
R1P	ribose 1-phosphate
R5P	ribose 5-phosphate
RCFFN	Richardson Centre for Functional Foods and Nutraceuticals
rpm	revolutions per minute
SAT	saturated fatty acid
SIMS	Sensory Integrated Management Software
SM	*semimembranosus*
ST	*semitendinosus*
TB	*triceps brachii*
TG	triglyceride

Chapter 1 – Introduction

Evidence suggests that meat constituted a substantial portion of the diet of even the earliest humans. Hunter and gatherers depended on the organized hunting of large animals such as bison and deer (Lawrie & Ledward, 2006). Although the consumption of bison has occurred for several years, in recent years, bison agribusiness has increased rapidly with approximately 200,000 bison being raised for livestock in Canada (Statistics Canada, 2006). There is also a notable shift in consumer behavior towards the purchase of alternative meat products. Bison is vastly becoming a popular meat choice due to its associated health benefits such as higher levels of unsaturated fatty acids compared to beef (Mihalopoulos et al., 2009). Several studies have observed the nutrient composition and health promoting benefits of bison meat, however the chemical analysis of water-soluble flavour precursors including reducing sugars, sugar phosphates, free amino acids, nucleotides, nucleosides and bases as well as lipids and their correlation with the sensory qualities of bison meat have not been extensively evaluated particularly during chilled storage conditioning at 4°C.

1.1 Conversion of Muscle to Meat

The present thesis focuses on raw meat and the effect of biochemical reactions on the formation of reducing sugars, sugar phosphates, free amino acids, nucleotides, nucleosides and bases as well as lipid flavour precursors as shown in Figure 1.1.1.

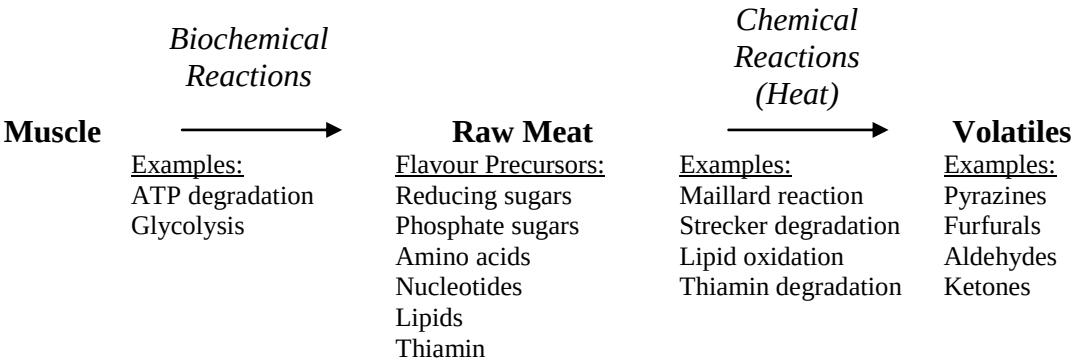

Figure 1.1.1 Conversion of muscle to meat. Development of flavour precursors via biochemical reactions in muscle post slaughter and their conversion to flavour volatile compounds during heating.

Meat often represents the muscle tissue of an animal. Muscles are made up of bundles of cells called fibers which are full of filaments made up of two proteins: actin and myosin. The conversion of muscle into meat is a complex process in which all mechanisms responsible for the development of meat qualities are interdependent (Oualia et al., 2006). Muscles are responsible for the majority of a carcass' weight (Hui et al., 2001). There are two types of musculature which are nonstriated and striated. Smooth muscles are nonstriated muscles, while cardiac and skeletal muscles are striated muscles. The muscle of interest in the current study is the skeletal muscle, specifically the *longissimus dorsi* (*LD*) muscle of *Bison bison*.

The concept of conversion of muscle to meat is complex as shown in Figure 1.1.2. Upon the slaughtering of an animal, from an industry stand point the animal undergoes

exsanguination, removal of hide, hair or feathers, evisceration and a final washing step which is completed within one hour post-slaughter.

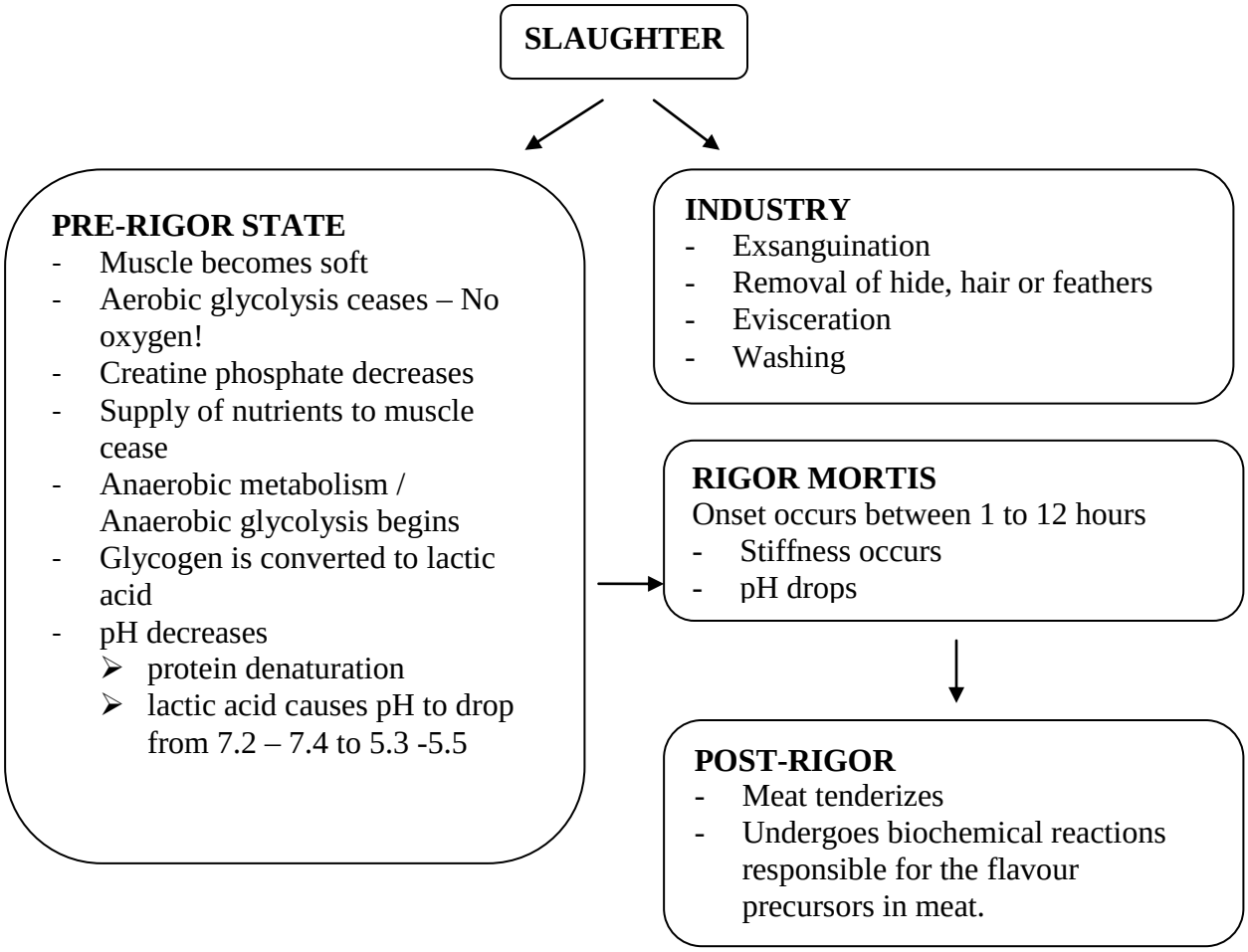

Figure 1.1.2 Conversion of muscle to meat: Post-slaughter steps adopted from the following references (Damodaran et al., 2008; Schreurs, 2000).

The animal undergoes three stages after death. The first step is the *pre-rigor* state in which the muscle becomes soft and aerobic glycolysis ceases due to the lack of oxygen. ATP and creatine phosphate decrease and the supply of nutrients to muscles ceases. Anaerobic metabolism and *post-mortem* glycolysis then begins. Glycogen is

converted to lactic acid. *Post-mortem* glycolysis is important to the overall quality of the meat. With no blood flow to carry the lactic acid away, the acid builds up in the muscle tissue. An acid content that is too high may result in meat that is pale and watery due to the loss of its water-binding ability whereas if the acid level is too low the meat will become tough and dry. In the *pre-rigor* state the pH decreases and causes protein denaturation while the lactic acid causes the pH to drop from between 7.2 and 7.4 to 5.3 and 5.5. The decrease in pH at this stage is very important for certain biochemical reactions to take place. The second stage is *rigor mortis*. *Rigor mortis* is a temporary process occurring during *post-mortem* glycolysis and is characterized by progressive stiffening of the muscle (Kinsmen, Kotula, & Breidenstein, 1994). The onset of *rigor mortis* occurs between 1 to 12 hours for most animals. During *rigor mortis*, stiffness occurs and the pH continues to drop. The third stage is *post-rigor* in which the meat tenderizes and the muscle continues to undergo biochemical reactions that are responsible for the flavour precursors in meat.

A study by Kim et al. (2000) observed the changes in the metabolic and pH concentrations during the 24 hour *post-mortem* period in two different muscle types *LD* and *psoas major* (*PM*) of five 21-month old Hanwoo bulls. The adenosine 5'-triphosphate (ATP) concentration drops drastically within the first 12 hours and then remains fairly constant for the *PM* muscle type whereas ATP in the *LD* muscle continues to gradually decline throughout the 24 hour *post-mortem* period. At the 24 hour period, both muscle types contained less than 2µmoles per gram of muscle of ATP. This can be explained by the initial amount of glycogen in each muscle when slaughtered. ATP is important for the commencement of selected biochemical reactions. Similar to the ATP

concentrations, the pH of both muscle types gradually decreased to a pH of approximately 5.6 to 5.8 over the 24 hour period (Kim et al., 2000).

1.2 Muscle composition

The average grass and grain-fed bison *triceps brachii* (shoulder clod), *longissimus thoracis*, *semimembranosus* (top round) and *gluteus medius* (top sirloin) muscle consists of roughly 75 percent moisture, 22 percent protein, 2 percent lipids and 1.2 percent ash (Marchello, 2001; Marchello et al., 1989; Marchello et al., 1998). Red meats normally contain iron, zinc, vitamin B_{12} and protein.

1.3 Flavour Precursors in Meat

Flavour is one of the most important sensory attributes for the overall acceptance of meat (Bryhni et al., 2002). It is important to note that flavour is described as the sensation caused by the properties of any substance taken into the mouth which stimulates one or both of the sense of taste and smell (Hart, 2007). The major flavour precursors of meat can be divided into two categories: (1) low molecular weight water-soluble compounds namely reducing sugars, sugar phosphates, free amino acids, nucleotides, nucleosides, bases and (2) lipids. Reducing sugars particularly monosaccharides are the most basic units of carbohydrates. Some monosaccharides have a sweet taste and are the building blocks of disaccharides and polysaccharides. Some can give rise to a number of isomeric forms which can have different chemical and physical properties but the same chemical formula. Reducing and phosphorylated sugars are limiting flavour precursors since even small changes in their natural concentration can have a significant impact on the eating quality of cooked meat. The importance of

reducing and phosphorylated sugars for meat flavour formation is via their contribution in the Maillard reaction in the presence of amino acids (Mottram, 1998). In chicken, ribose, a five carbon reducing sugar, is particularly important for flavour generation (Aliani et al., 2008) . Results demonstrate that an increase of only 2 to 4 fold in ribose concentration (25 mg/100g wet weight) was sufficient to significantly increase the desirable aroma and flavour of cooked meat (Aliani & Farmer, 2005a).

Amino acids are molecules containing an amine group and a side-chain that varies between different amino acids. The key elements of an amino acid are carbon, hydrogen, oxygen and nitrogen. Amino acids are the structural units that make up proteins and are broken into essential and nonessential amino acids in the human diet. Some of the essential amino acids include: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, whereas some nonessential amino acids that may be essential in some cases include: alanine, cysteine, glutamine, proline, serine, tyrosine, arginine, ornithine, etc. (Fürst & Stehle, 2004). Sulfur containing amino acids are important flavour precursors due to their participation in the Maillard reaction. Cysteine is shown to be an important sulfur containing compound that when present in the Maillard reaction helps generate desirable key volatiles in meat such as a roasty aroma and flavour (Mottram & Nobrega, 1998).

Nucleotides are composed of a nitrogenous base, a five-carbon sugar and one to three phosphate groups. Phosphate groups form bonds with either the 2, 3, or 5-carbon of the sugar. Nucleotides can contain either a purine or a pyrimidine base. An example of a nucleotide is ATP or inosine 5’-monophosphate (IMP). The nucleobase and sugar

(ribose) comprise a nucleoside, for example cytidine or inosine. An example of a base is hypoxanthine.

It has been recognized since research in the 1950s and 1960s that lipids are important precursors of the characteristic aroma of cooked meats due to their thermal degradation (Mottram, 1998). Lipids encompass molecules such as fatty acids and their derivatives including phospholipids as well as other sterol-containing metabolites such as cholesterol. Triglycerides are esters derived from a glycerol and three fatty acids. Thermal oxidation, lipid oxidation and/or degradation contribute to the flavour of meat in addition to the adipose tissue which may contribute to the overall juiciness of the meat (Enser, 2001). In fresh meat, lipids also contribute to oxidative rancidity (Enser, 2001) as well as both desirable and undesirable volatiles upon heating (Mottram, 1987).

1.4 Chemical Reactions

Uncooked meat has little or no aroma and only a blood-like taste (Gwartney, 2005). When meat is cooked, the individual wound-up coils of protein molecules that are formed and held together by bonds are broken and the coils unwind. Heat also shrinks muscle fibers which cause water to be released and protein molecules to recoagulate. This process is called denaturation since the structure of the protein changes. The characteristic meat flavour is formed during the cooking process when non-volatile flavour precursors react through a series of chemical reactions. The Maillard reaction is one of the most important non-enzymatic chemical reactions that occur between an amino group of an amine, amino acid, peptide or protein and a carbonyl group of a reducing sugar during heating processes.

The Maillard reaction was first described in the 1910s and was named after Louis-Camille Maillard. The Maillard reaction is responsible for the brown pigments, desirable flavours and nutritive changes in meat. When the reactive carbonyl group of sugar reacts with the amino group of the amino acid a range of aromas and flavours are formed. The browning reactions that occur in meat cause a roasted and seared flavour. High temperature, intermediate moisture levels and alkaline conditions all promote the Maillard reaction but browning reactions do not occur until all surface water is vaporized (Albumen, 1982).

Strecker degradation is another chemical reaction that involves the oxidative deamination and carboxylation of an α-amino acid in the presence of a dicarbonyl compound (Mottram, 1991). This leads to the formation of an aldehyde containing one fewer carbon atom than the original amino acid, and an α-aminoketone. The aminoketones are important intermediates in the formation of several classes of heterocyclic compounds including pyrazines and thiazoles. In the Strecker degradation of cysteine, hydrogen sulfide, ammonia and acetaldehyde are formed, as well as the expected Strecker aldehyde mercaptoacetaldehyde and an α-aminoketone (Mottram, 1991).

Lipids can contribute to meat flavour in many ways such as (1) thermal oxidation of lipids which is another major reaction during heating of meat that results in flavour active compounds that contribute to meat flavour (Mottram, 1996; Mottram, 1998), (2) lipids may react with other components in tissues which creates other flavour compounds, (3) lipids may also act as a solvent for several aroma compounds such as

sulfur and potentially minimize their aroma potential to help produce more desirable meat flavours (Mottram, 1996; Wasserman, 1972).

1.5 Chilled Storage Conditioning

Chilled storage conditioning influences the formation of different flavour precursors and has been shown to be crucial to the eating quality of different types of meat (Koutsidis et al., 2008a; Mottram & Nobrega, 1998). Under hygienic conditions, meat can be stored at or above its freeing point (-1.5°C) for a few weeks without spoilage, however as the muscle pigment myoglobin denatures, its iron oxidizes, which may cause a brown discoloration near the surface of the meat (Lawrie & Ledward, 2006). With prolonged storage, meat will undergo an aging process that can increase its tenderness and flavour. Overtime, muscle proteins will denature with the exception of collagen and elastin in connective tissue and likely undergo proteolysis. Flavour precursors are formed by enzymatic and microbial changes in *post-mortem* muscle. Microbial changes are important, however they are not investigated in this research. Enzymatic changes are affected by conditions such as temperature and pH which will alter the flavour profile of the meat. In order to evaluate how *post-mortem* conditioning may affect flavour formation in meat, changes in nucleotide degradation products, free amino acids, reducing sugars and sugar phosphates as well as lipid concentrations in bison *LD* muscle stored at 4ºC.

This is the first study to investigate the effect of chilled storage conditioning on key flavour precursors in bison meat and to correlate these chemical results including reducing sugars and sugar phosphates, free amino acids, nucleotides, nucleosides and

bases as well as lipids to those observed during sensory evaluations of cooked bison meat stored at 4°C for 2, 4, 8, 15 and 21 days.

In a recent study, the *longissimus lumboram* (LL) muscle of 16 Charolais steers raised on a concentrate diet was analyzed at 1, 3, 7, 14 and 21 days for nucleotide, sugar, amino acid and free phosphate analysis (Koutsidis et al., 2008a). The conclusion of this study indicated that the most significant changes occurred in the increased concentrations of the water-soluble flavour precursors of ribose, methionine and cysteine, which are all important in the potent meat flavour volatiles in model systems upon heating (Koutsidis et al., 2008a). After 21 days of conditioning, the increase in total amino acids was greater than that observed for sugar and sugar phosphates.

1.6 Hypothesis

The hypothesis of this thesis was:

The concentration of key flavour precursors generated by different biochemical reactions will be altered during chilled storage at 4ºC. The concentration changes and overall balance of key flavour precursors during *post-mortem* conditioning will impact the eating quality of cooked bison meat.

1.7 Objectives

The objectives of this thesis were threefold:

(1) To elucidate the effect of *post-mortem* conditioning of *Bison bison LD* muscle during chilled storage at 4ºC on the formation of selected flavour precursors in the muscle.

(2) To evaluate the effect of chilled storage conditioning at 4°C on the sensory quality of cooked bison meat.

(3) To correlate sensory evaluation results to the chemical analysis of flavour precursors in *post-mortem LD* muscles of *Bison bison* during chilled storage conditioning at 4°C.

Chapter 2 - Experimental Design

2.1 Choice of Animal and Muscle Type

In recent years, bison agribusiness has increased rapidly with approximately 200,000 bison being raised for livestock in Canada (Statistics Canada, 2006). There is also a notable shift in consumer behavior towards the purchase of alternative meat products. A 2004 telephone interview survey by CROP Research Marketing, looked at the meat product consumption habits of North Americans and observed that out of 301 Canadians (68% female and 32% males between 18 and over 65 years of age), more than 6 in 10 respondents (including the United States and Mexico data) are often or sometimes on the lookout for new food products. Almost 38% of Canadians (Quebec, Ontario, Manitoba, Saskatchewan, Alberta and British Columbia) consume specialty meats. Among all specialty meats, bison is mentioned most often (47%), with approximately 64% of Canadian (n=231) respondents that indicated they have heard of bison meat. Approximately 92% of Canadian households consume meat, while bison meat consumption frequency indicates that out of 278 Canadians, 5% ate bison at least once a month, 18% less than once a month while 77% of Canadians have never had bison meat. Of those respondents that have consumed bison meat, 47% and 29% considered bison meat to be just as nutritious or more nutritious than traditional meats respectively, while 42% and 74% thought bison meat had fewer calories and less fat than traditional meat, respectively and 54% found the quality of bison meat to be about 54% the same as traditional meat products (CROP, 2004). Bison meat ultimately benefits from a high level of awareness in Canada and at the forefront of choosing alternative meats, bison has been

deemed to have fewer calories and lower fat content (Driskell et al., 1997; McClenahan & Driskell, 2002) when compared to beef (Rule et al., 2002).

Many studies have looked at the nutritional composition, cooking methods (Juárez et al., 2010; McClenahan et al., 2001), effect of food preparation and cooking treatments via aging, heat treatment, mechanical and electrical manipulation as well as the effect of additives such as antioxidants (Sahoo & Anjaneyulu, 1997), binders (Devadason et al., 2010; Modi et al., 2004) and tenderizers (Dhanda et al., 2002; Naveena et al., 2004) on sensory qualities of bison meat. At this point, no studies have looked at the small molecular water-soluble compounds such as amino acids, reducing sugars, nucleotides, nucleosides and bases and their contribution to the formation of bison meat flavour particularly during chilled storage conditioning at 4°C. The concentration of flavour precursors in meat is known to be affected by factors such as diet, breed, species and *post-mortem* conditioning (Dwivedi, 1975). For the purpose of this research, the *longissimus dorsi (LD)* (ribeye) muscle was used because it is a popular muscle type consumed and has been used in many bison studies to date. Figure 2.1.1 shows the different muscle cuts. The rib spans from the sixth rib to the twelfth. The *longissimus dorsi* muscle is the longest subdivision of the back muscle groups. Meat from bison (*Bison bison*) also known as North American buffalo, is gaining acceptability as an alternative meat source (McClenahan et al., 2001) and has been used for this research.

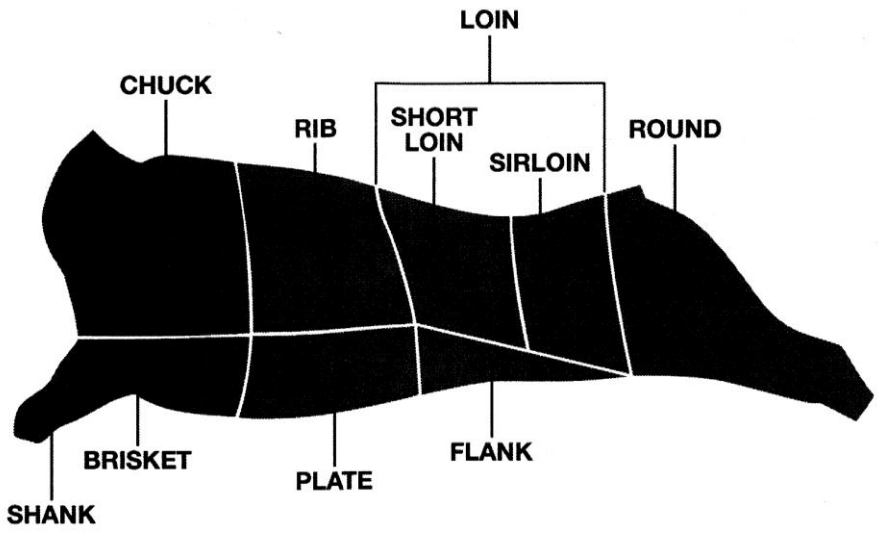

Figure 2.1.1 Bison muscle cuts (Beef University, 2011).

2.2 Meat Samples

The *LD* muscle from six grain-fed heifers (approximately two years old) was provided by the Manitoba Bison Association. Bison were slaughtered according to Canadian Food Inspection Agency (CFIA) regulations and the *LD* muscles were removed two days post-slaughter at Winkler Farms (Winkler, MB, Canada) after which they were delivered to Preferred Meats (Winnipeg, MB, Canada) in separate vacuum packed bags. The *LD* muscle (Figure 2.1.2) was pre-cut into steaks of 2.5cm thickness and vacuum packed at the Weston Sensory Laboratory at the University of Manitoba in Food Saver 11-inch x 18 feet vacuum packaging bags (Zellers, Canada) that were cut and sealed with a Food Saver Advanced Design V2490 Home Vacuum Packaging System (London Drugs, Canada). Steaks were held at 4°C for 2, 4, 8, 15 and 21 days post-slaughter after which water soluble and lipid soluble extractions as well as sensory evaluations took place. One bison steak from each animal was used for all extractions.

Figure 2.1.2 Bison *longissimus dorsi* muscle.

2.3 Shelf-life of Fresh Meat

The shelf-life of fresh meat is of huge importance in the retail marketplace. Shelf-life is the amount of time that passes before a meat product becomes unpalatable or unfit for human consumption due to growth of spoilage organisms (Delmore, 2009). Although this research does not focus on microbiological growth, appropriate procedures were followed in regards to meat handling, storage temperature and length of storage to ensure microbiological safety of fresh bison meat for both chemical and sensory evaluation analysis. Shelf-life properties may include appearance, texture, flavour, colour and nutritive value (Singh & Singh, 2005).

The most effective practice for improving the safety and quality of meat is storage temperature (Koutsoumanis & Taoukis, 2005). Refrigeration temperatures of 4°C was used to store bison meat for 2, 4, 8, 15 and 21 days for this study, which has been shown to extend the shelf life of meat, however meat can still spoil as a result of aerobic, psychotropic bacteria which are defined as bacteria that are capable of growth in cold environment (Lambert et al., 1991). Vacuum-packaging of fresh and chilled bison meat

was used to modify the internal environment in hopes to extend shelf life and help ensure the quality of fresh meat. Vacuum packaging was accomplished using a vacuum packaging bag with very low moisture and oxygen transmission rates. Oxygen was removed via a vacuum chamber and package was heat sealed to keep in freshness and seal out environmental determinants such as oxygen that help bacteria grow. In wholesale, fresh beef primals have a 4°C shelf life of 38 days as long as it is vacuum packaged properly (American Meat Institute Foundation, 2011).

The composition of meat can also influence microbial spoilage. Protein and fat do not change and are not substrates for microbial attack during the onset of rigor, whereas bacteria often use low-molecular weight substances formed during *post-mortem* glycolysis. For example, glucose is a substrate used for growth of *Alteromonas putrefaciens, Brochothrix thermosphacta, Enterobacter* and *Lactobacillus* micro-organisms during anaerobic conditions (Gill, 1986).

Microbial spoilage and growth of micro-organisms was not a priority for this study, however it was important to address and ensure proper safety procedures at all times when handling, storing and especially when cooking fresh meat products for human consumption. Conditioning times for this study were carefully considered. The early conditioning time of 2 days was established based on the regulations by the CFIA that would not allow the carcass to be cut until the bison carcass reached mandatory pH level. The final conditioning time of 21 days was established on the basis that the meat is still acceptable and meets microbiological safety levels associated with fresh meats.

2.4 Extraction Methods for Flavour Precursors in Meat

2.4.1 Water-soluble extraction for flavour precursors

The water-soluble extraction method used for bison analysis of free amino acids, reducing sugars, sugar phosphates, nucleotides, nucleosides and bases was adapted from (Koutsidis et al., 2008b) and is outlined in Figure 2.1.3. Bison steaks were removed from storage at 4°C and weighed on a TP-3101 scale (Denver Instrument, USA) and recorded. Steaks were cut into small cubes using a razor, all visible fat was removed and 30 grams of meat was homogenized using a Hamilton Beach Chef Prep (Sears, Canada) commercial chopper. The remainder of the steak was vacuum packaged in Food Saver 11-inch x 18 feet vacuum packaging bags (Zellers, Canada) that were cut and sealed with a Food Saver Advanced Design V2490 Home Vacuum Packaging System (London Drugs, Canada) and stored at 4°C. Meat was homogenized for 15 pulses or 15 seconds, homogenization time and date was recorded. The pH of homogenized meat was recorded in duplicate for accuracy using PICCOLO Plus ATC pH/°C meter (Hanna Instruments, Portugal). The pH of homogenized meat was 4.98±0.15 for bison *LD* muscle at storage day 2 and pH did not change throughout conditioning. All equipment was washed after each steak extraction. Three grams of meat was accurately weighed on a TP-3101 scale and transferred into 28 ml Nalgene Oak Ridge centrifuge tubes (Rochester, NY, USA). The remainder of the homogenized meat was vacuum packed and placed at -80ºC. The addition of 10 ml of cold deionized water and internal standards including 300 µl of rhamnose (2 mg/ml), 300 µl of norvaline (1.5 mg/ml) and 400 µl of cytidine (1 mg/ml) which were prepared in deionized water for sugar, amino acid and nucleotide analysis, respectively. Tubes were then capped and shaken by hand for 5 minutes followed by

centrifugation for 30 minutes at 3,000*g* at 4ºC in an AccuSpin™ 3R centrifuge (Fisher Scientific, Ottawa, ON, Canada). The supernatant was decanted and filtered under gravity through Whatman 54 filter papers (Fisher Scientific) using short stem Kimble Chase funnels (KIMAX, Mexico) into 50 ml polypropylene Corning tubes and the residues were re-extracted with 5 ml of cold deionized water and placed in the centrifuge for 15 minutes at 3,000*g* at 4ºC. The supernatant was then combined with the previous supernatant and filtered again through Whatman 54 filter papers into new 50 ml polypropylene Corning tubes to remove any fat and/or tissue particles. To complete the water-soluble extraction process, 10 ml of filtrate was transferred to a 15 ml Amicon Ultra (3000 Da molecular weight cut-off) ultrafiltration tube (Millipore Corporation, Bedford, MA, USA) and placed in centrifuge for 4 hours at 3,000*g* at 4ºC. All water-soluble extractions were performed in duplicate and the filtrates were stored at -80ºC until analyzed.

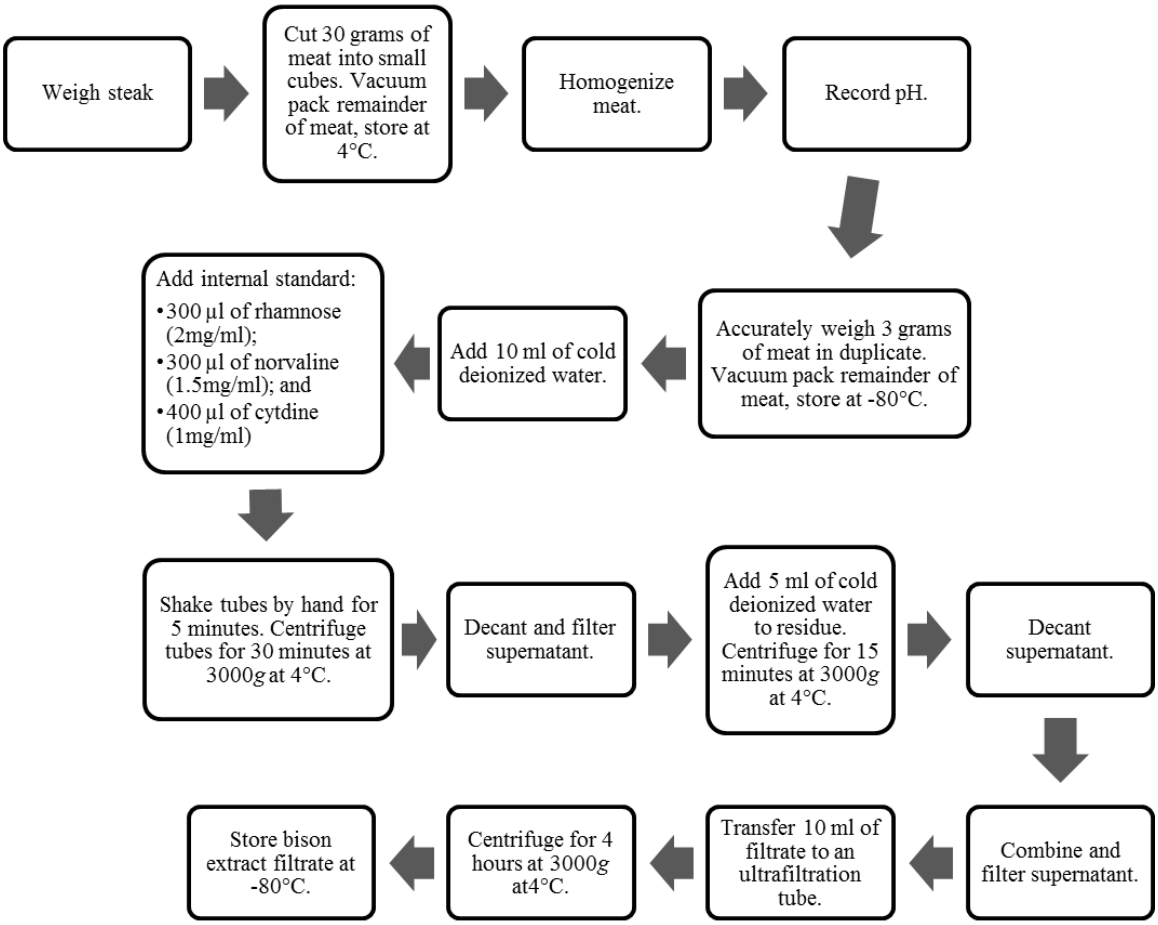

Figure 2.1.3 Flow chart of water-soluble extraction of bison *LD* muscle.

2.4.2 Lipid-soluble extraction for flavour precursors

The lipid-soluble extraction method used for analysis of free fatty acids (FFA), triglycerides (TG), phospholipids (PL) and cholesterol was from Folch et al. (1957) and is outlined in Figure 2.1.4. One gram of homogenized meat (described in section 2.2) in duplicate was accurately weighed and transferred to 50 ml Pyrex tubes (Fisher Scientific) followed by 4 ml of 0.025% anhydrous calcium chloride ($CaCl_2$) solution. Calcium chloride was 96% pure and was purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). The tissue and $CaCl_2$ was then homogenized using PT 2100 polytron (Kinematic, Switzerland) for approximately 5-10 seconds. Polytron was rinsed 3 times with distilled water and any tissue was removed from blades between samples to avoid cross contamination. A total of 20 ml of ≥99.9% chloroform and methanol (C:M) (2:1) purchased from Sigma-Aldrich Canada Ltd. was added to tubes; vortexed using a Maxi Mix II (Thermo Scientific, Dubuque, IA, USA) for 1 minute and centrifuged in an Allegra™ 6 centrifuge (Beckman Coulter Canada Inc., Ontario, Canada) for 12 minutes at 2000 revolutions per minute (rpm) at room temperature. Samples were then removed and placed at 4ºC for 1 to 2 hours to achieve good phase separation. The upper phase consisting of methanol and water was transferred into 30 ml Pyrex tubes using glass pipets and placed a -20ºC, while the lower phase consisting of chloroform, neutral lipids, phospholipids and cholesterol was transferred using glass pipets into pre-weighed 30 ml Pyrex methylated tubes without caps. Lower phase tubes were then dried down under nitrogen using a N-EVAP™111 nitrogen evaporator (Organomation Associates Inc., Berlin, MA, USA) with low heat (45-50ºC) until completely dried. Tubes were then weighed and total lipids were calculated by subtracting the final weight of tube without

caps containing lipid from initial weight of tube without caps. To ensure no tissue was incorporated into the weight of total lipids, a final rinsing step was added and involved the addition of C:M (2:1) to tubes containing lipids. Sides of tubes were rinsed and lipids were transferred to 15 ml methylated pre-weighed Pyrex tubes, followed by a second rinse with C:M (2:1) and was once again added to the same smaller methylated tube. Tubes were dried down under nitrogen, weighed and total lipids were re-calculated and 1 ml of C:M (1:1) was added to each tube. The crimped-part of tubes was then taped with teflon tape (Rona, Winnipeg, MB, Canada) to avoid evaporation of chloroform and samples were then stored at -80ºC. At a later date, tubes were re-dried under nitrogren, weighed and total lipids were re-calculated. C:M (1:1) was then added at a concentration of 25 mg/ml. All lipid extractions were performed in duplicate and stored at -80ºC until analysis.

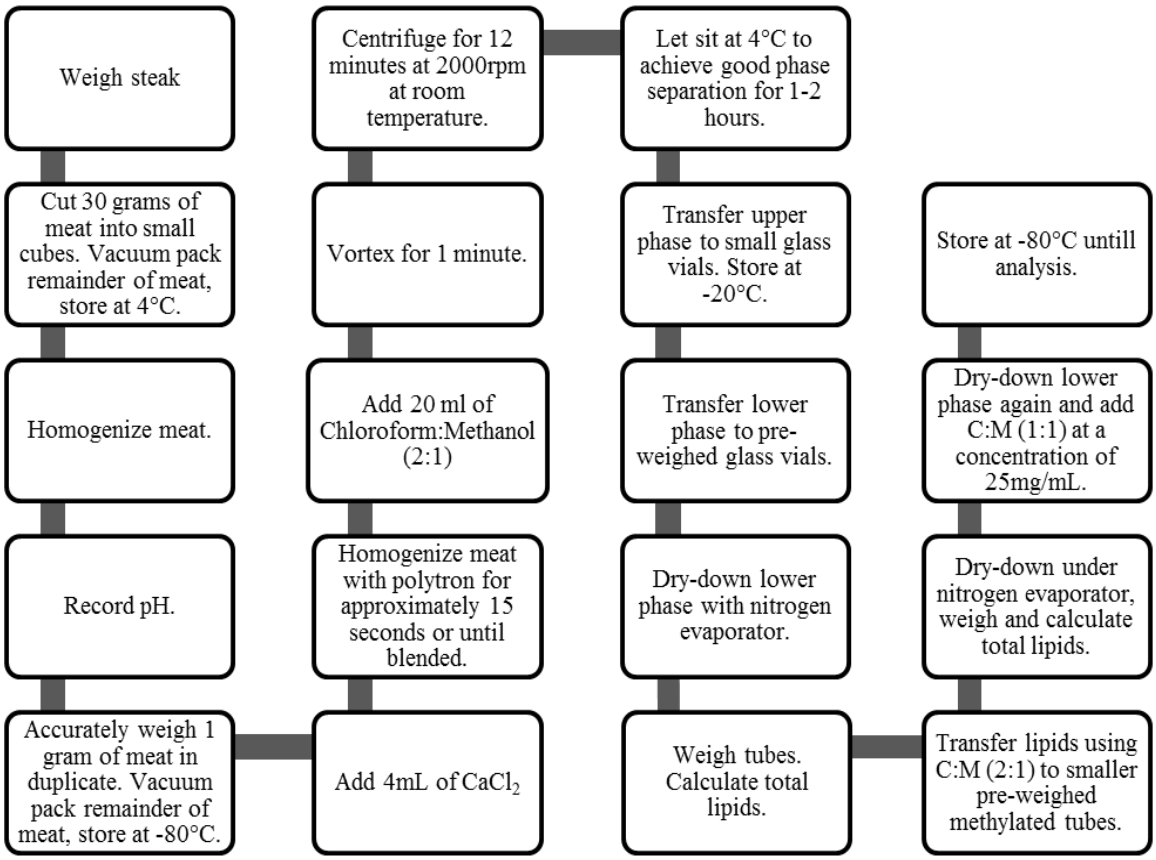

Figure 2.1.4 Flow chart of lipid-soluble extraction of bison *LD* muscle.

Chapter 3 - Sensory Evaluation

With a shift in consumer behavior towards purchasing alternative meat products such as bison as discussed in section 2.1, investigating the sensory qualities of bison meat is important for both producers and consumers. Flavour is one of the most important sensory attributes to the overall acceptance of meat (Bryhni et al., 2002). Meat flavour is thermally derived (Mottram, 1998) and is formed during the cooking process when non-volatile flavour precursors react through a series of chemical reactions. The Maillard reaction is one of the most important non-enzymatic chemical reactions that occurs between amino acids and reducing sugars during heating processes (Mottram, 1998). The Maillard reaction is responsible for the brown pigments and desirable meaty flavours in cooked meat (Koutsidis et al., 2008b; Mottram, 1998). While the flavour of cooked meat is influenced by compounds contributing to the sense of taste, it is the volatile compounds formed during cooking that determine the aroma attributes and contribute to the characteristic flavours of meat (Mottram, 1998).

The amount and nature of precursors present in meat depends on several factors including species, diet, breed and *post-mortem* treatment including ageing and conditioning (Koutsidis et al., 2008a). Chilled storage conditioning has been shown to be crucial to the eating quality of different types of meat such as beef (Koutsidis et al., 2008a) and pork (Meinert et al., 2007). Several studies have demonstrated the importance of conditioning on the enhancement of certain meat sensory attributes. Miller et al. (1997) concluded that ageing of beef for 14 days compared to 7 days increased flavour intensity as measured by trained panelists (Miller et al., 1997). Significant changes in flavour attributes are related to extended *post-mortem* conditioning and an increase in

flavour intensity can be attributed to the accumulation of water-soluble flavour precursors (Campo et al., 1999). Few studies have correlated the chemical analysis of flavour precursors with sensory evaluation analysis and quality changes related to flavour particularly in bison meat.

The present study aimed to evaluate the effect of chilled storage conditioning at 4ºC on the sensory quality of cooked bison meat and to further correlate sensory evaluation results to the chemical analysis of flavour precursors in *post-mortem LD* muscle of bison during chilled storage conditioning at 4°C.

3.1 Meat Samples

Preparation of meat samples used for sensory evaluation is outlined in section 2.2. After steaks were held at 4°C for 2, 4, 8, 15 and 21 days post-slaughter after which they were frozen at -20°C to test the effect of storage day conditioning on the eating quality of bison meat.

3.2 Ethics Approval

Volunteers were recruited from staff and student populations according to the Joint-Faculty Research Ethics Board at the University of Manitoba. The ethics approval certificate is located in Appendix A.

3.3 Maintenance of Confidentiality

Confidentiality was maintained by assigning a random number to all panelists. The sheet that lists the subjects and their number was only accessible to the principal researcher and his assistant. Once all sessions were completed the list was shredded so

that final data would only be recorded using panelist numbers to facilitate data entry. Published data will not be connected with any names of individuals but reported as mean values only. All personal information provided by the panelists will be stored in a locked cabinet and shredded after five years from the date of ethics approval or publication of the research, whichever comes first.

3.4 Quantitative Descriptive Analysis

Sensory analysis was conducted using a quantitative descriptive analysis method. Quantitative descriptive analysis (QDA) is one of the main descriptive analysis techniques in sensory evaluation (Stone & Sidel, 2004). The method is based on a panelist's ability to verbalize perceptions of a product in a reliable manner and involves a training procedure, development and use of sensory language, and the scoring of products on repeated trials to obtain a complete quantitative description (Stone, 1992). Panelists for the bison sensory evaluation were trained to employ an unstructured 15cm line scale to score the selected sensory attributes, where 0 indicated a low and 15 indicated a high attribute intensity. Appendix B is an example of an unstructured 15cm line scale ballot.

3.5 Materials and Methods

3.5.1 Recruitment of panelists

Panelists were recruited for the bison tasting through email (Appendix C). All members of the Faculty of Human Ecology (FHE) and Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba were sent an email containing a recruitment letter (Appendix D), written consent form (Appendix E) and questionnaire (Appendix F) as attachments. Posters were also posted around the FHE as

well as at the RCFFN at the University of Manitoba. Word of mouth was also encouraged. No previous participation in sensory evaluation panels was required by volunteers. All volunteers were made aware that they would be trained to evaluate aroma, flavour and texture attributes of bison steaks over a course of six to eight training and three evaluation sessions that would run approximately 45 minutes each. Only volunteers that were available, interested and were not allergic to any food products used in the study were selected as a panelist. Panelists consisted of one male and seven females between the ages of 20 to 35. All panelists were compensated for their participation with $50 gift cards for the University of Manitoba bookstore, under the condition that they participated in every training and evaluation session.

3.5.2 Sample and standard preparation

Before sensory analysis, five bison steaks representing the five storage days were randomly selected from steaks available from six different bisons and thawed at 4°C on a tray overnight for approximately 15 hours. Steaks were cut into 1.5cm cubes (approximately 6.92±0.50 grams raw weight) and placed in 6.4cm x 2cm aluminum foilware BakeKing Tart Forms (Sobeys, Canada), which were capped and sealed with pre-cut aluminum foil as shown in Figure 3.1.1a. All samples were labeled with a random three-digit code (see Figure 3.1.1b) using SIMS 2000 Version 6.0 (2009) computerized sensory software (Sensory Integrated Management Software, Morristown, NJ, USA). Samples were then randomly placed on 43.8 x 29.2 x 2.5cm baking sheets and held at 4°C for approximately 1½ hours before cooking. For sensory evaluation, each tray had two reference samples that were used for internal temperature testing of samples during cooking.

a) b)

Figure 3.1.1 a) Cut raw bison samples b) Bison samples prior to cooking

3.5.3 Sample cooking and holding

Bison samples were cooked in a Frigidaire Professional Series Even Cook conventional oven on the third rack level from the top. The oven was turned on approximately 15 minutes before cooking in order to reach and stabilize at the desired cooking temperature of 121°C (250°F). One tray was placed in the oven at a time. Samples were cooked at 121°C for 10 minutes to an internal temperature of 74.7±4.4°C. Internal temperature was tested using a Type K thermometer (Fisher Scientific, Ottawa, ON, Canada). According to Health Canada (2010), an internal temperature of 71ºC is considered to be medium while 77ºC is considered well done (Health Canada, 2010). Pink and red coloured meat and pink juices were observed in bison samples with an internal temperature less than 70ºC, therefore an internal temperature closer to 75ºC with slight pink coloured meat and clear juices was considered optimal for the cooked bison samples without reducing meat tenderness.

Corning PC-300 model hotplates (Fisher Scientific) were used to hold a maximum of four samples at 50°C until randomly sampled by panelists. All samples were analyzed immediately after preparation was complete.

3.5.4 Training of panelists

During six training sessions of 45 minutes each, panelists worked together as a group to identify, define attributes and determine appropriate standards that clarify these attributes in bison under guidance of an experienced group leader. All disagreements amongst panelists were addressed and standards were altered accordingly until an agreement was met amongst the panelists. Panelists agreed on the standards that represented the attributes and their definitions shown in Appendix G. A total of four aroma, four flavour and six texture attributes were determined amongst the group to identify and define cooked bison attributes based on the standards and meat samples used during training.

3.5.5 Sensory evaluation

Three evaluation days were held to evaluate the intensity of four aroma, four flavour and six texture attributes of cooked bison meat representing the five different bison samples that were randomly selected and represent the five different conditioning days of 2, 4, 8, 15 and 21 days. Panelists were seated in individual partitioned work stations at the Weston Sensory Laboratory at the University of Manitoba as shown in Figure 3.1.2. All stations were equipped with SIMS 2000 Version 6.0 (2009) computerized sensory software, while light from incandescent bulbs directed through red opaque plastic masked color differences in samples. Filtered water at room temperature

was provided in each work station for cleansing the palate between samples and white cotton gloves were provided in case samples were slightly warm. Samples were randomly selected using SIMS 2000 Version 6.0 (2009) computerized sensory software and were placed on hot plates through sliding doors in each work station when they came out of the oven.

Figure 3.1.2 Sensory panel work stations.

3.5.6 Statistical analysis

Two-factor mixed model (panelist – random effect; storage day – fixed effect) ANOVA was performed using SPSS (Version 19) and PanelCheck V1.4.0 software programs. Mean storage day differences were determined using least significant difference (LSD) with a significance level of $P<0.05$. According to Lawless and Heymann (1999), the 2-way ANOVA is an appropriate statistic for the use in sensory analysis when all panelists rate all products (Lawless & Heymann, 1999).

3.6 Results and Discussion

The F-values of the sensory attributes for the storage day effect are shown in Figure 3.1.3. Baking of bison *LD* muscle at 121°C showed a significant increase in

vinegar/sour aroma, initial tenderness, juiciness and overall tenderness and a significant decrease in connective tissue and chewiness attributes at a significance level of P<0.05 and P<0.001 between storage days. Mean storage day differences using LSD were observed between 2 and 15 days for the above aroma and texture attributes (see Table 3.1.1). Initial tenderness, juiciness and overall tenderness significantly increased from storage day 2 at 6.6±3.2, 6.6±2.8 and 5.9±3.4 to 10.9±2.5, 9.7±2.8 and 10.4±2.5 at storage day 15 respectively, followed by a significant decrease in these attributes at storage day 21. No significant differences in beef, brothy/salty and oily/fatty aromas, beef, sour, salty and oily/fatty flavours and oily mouth coating were found between storage days of bison meat.

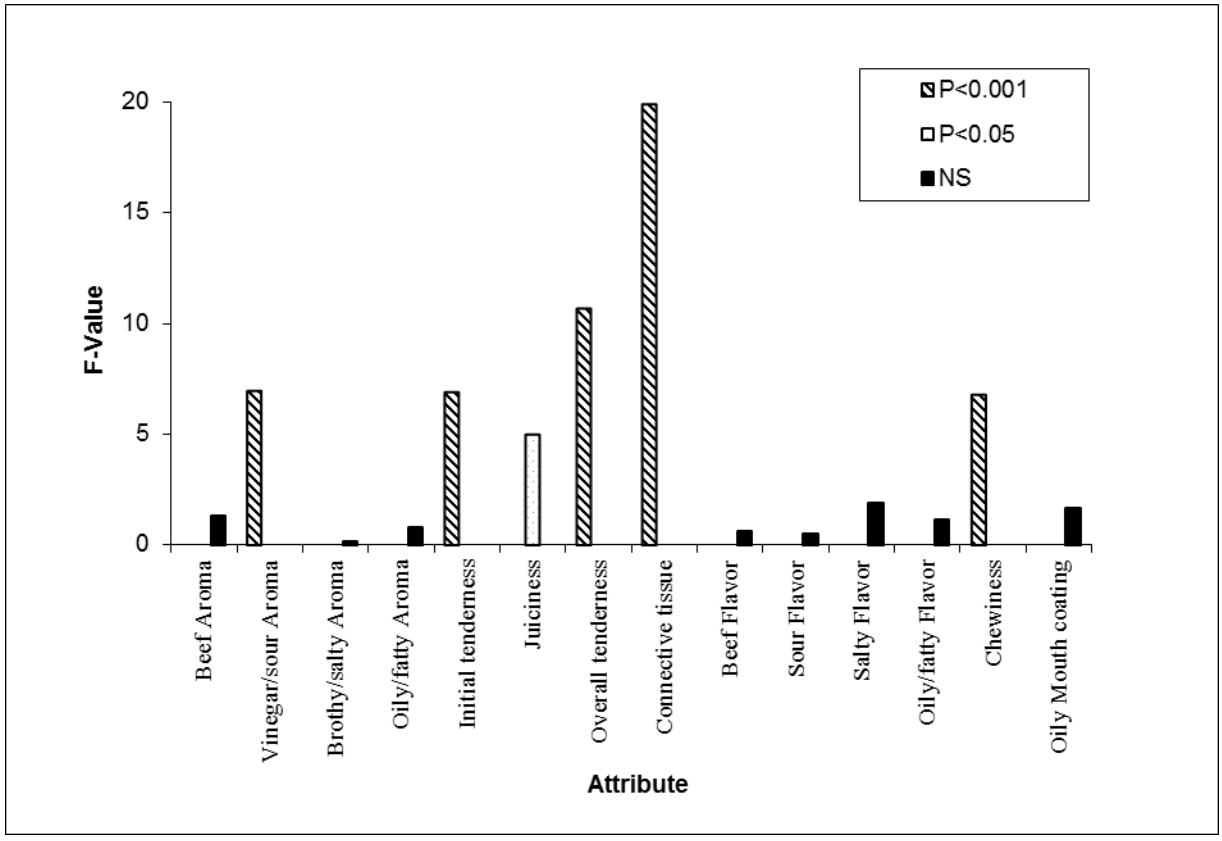

Figure 3.1.3 Storage day effect on sensory attributes of bison *LD* muscle.

Table 3.1.1

Storage day effect on sensory characteristics of bison meat.

Attributes	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
Beef Aroma	9.3±2.6	8.1±2.6	7.8±3.0	8.3±3.0	9.2±2.6
Vinegar/Sour Aroma	**5.7±2.5[a]**	**6.4±2.7[ab]**	**6.4±3.2[ab]**	**7.6±3.0[b]**	**6.9±2.4[b]**
Brothy/Salty Aroma	6.8±2.9	6.8±2.9	7.1±3.1	6.9±3.2	7.0±2.9
Oily/Fatty Aroma	7.8±2.9	6.8±3.0	7.0±2.9	7.0±3.4	6.8±2.7
Beef Flavour	8.8±3.0	8.5±2.6	8.6±2.8	9.0±2.3	9.6±1.7
Sour Flavour	6.4±3.6	7.0±3.4	6.8±3.3	6.9±3.0	6.9±2.9
Salty Flavour	5.6±2.9	5.4±2.5	6.0±2.8	6.4±3.1	6.0±2.6
Oily/Fatty Flavour	6.4±3.4	6.2±2.6	6.0±2.8	7.3±3.0	6.4±3.1
Initial Tenderness	**6.6±3.2[a]**	**7.6±2.6[ab]**	**9.2±3.3[b]**	**10.9±2.5[c]**	**8.4±3.4[b]**
Juiciness	**6.6±2.8[a]**	**8.4±2.4[bc]**	**9.0±2.5[bc]**	**9.7±2.8[c]**	**8.2±2.7[b]**
Overall Tenderness	**5.9±3.4[a]**	**7.8±2.9[b]**	**8.3±2.9[b]**	**10.4±2.5[c]**	**8.6±2.9[b]**
Connective Tissue	**8.2±3.5[a]**	**7.0±3.0[ab]**	**7.1±3.4[ab]**	**4.2±3.0[c]**	**5.7±3.2[bc]**
Chewiness	**9.8±3.3[a]**	**8.7±2.8[ab]**	**8.3±3.6[ab]**	**5.2±7.0[c]**	**7.1±3.7[b]**
Oily Mouth Coating	5.5±2.7	5.7±3.1	5.7±3.3	7.0±2.9	6.5±3.7

Note. Values are means of 8 panelists in triplicate ± standard deviation.

[1]Different letters in the same row indicates a significant difference at $\rho<0.05$.

Results shown in Figure 3.1.4 demonstrate the panelist effect on sensory attributes of bison meat. Significant differences in F-values in panelists for most attributes except for initial tenderness and juiciness are shown. These differences can be partly explained by inconsistencies between panelists ranking attributes in different orders or panelists

ranking attributes on different sections of the unstructured 15cm line scale. The panelist/storage day interaction shown in Figure 3.1.5 shows no significant difference in all attributes except beef flavour at a significance level of P<0.05. This difference has been associated with one panelist that ranked the attribute higher than other panelists. Removing this panelist from the results was not an option as this panelist contributed results that were in range with other panelists for all other attributes.

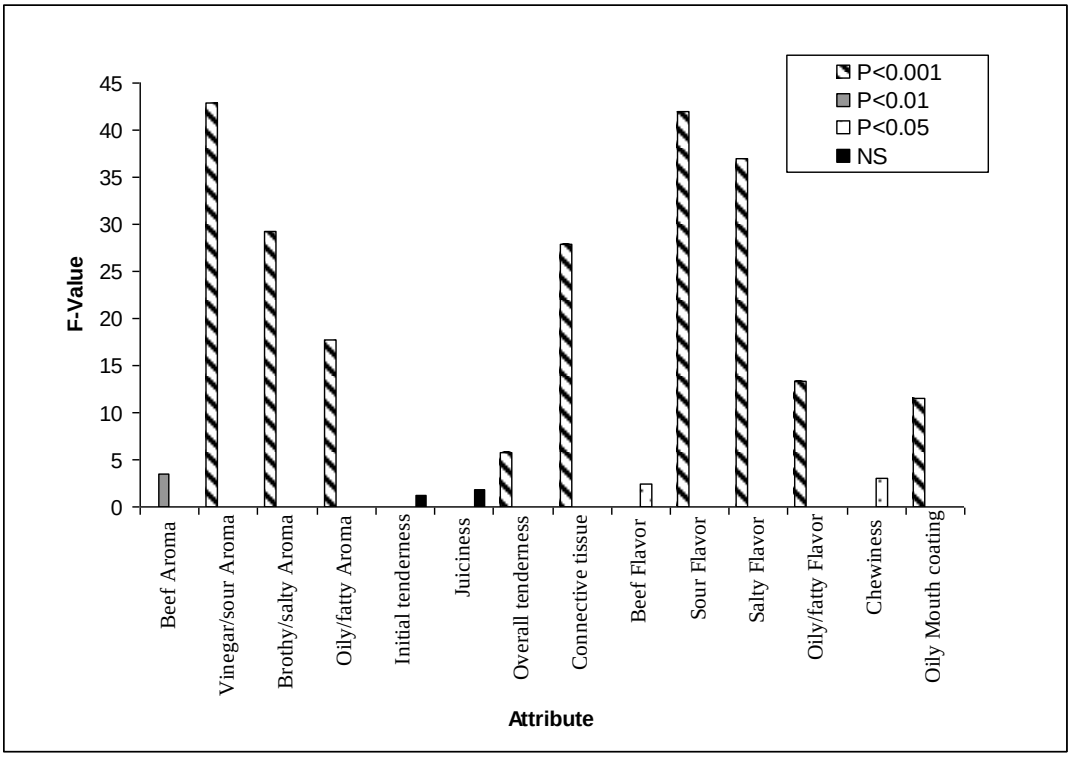

Figure 3.1.4 Panelist effect on sensory attributes of bison *LD* muscle.

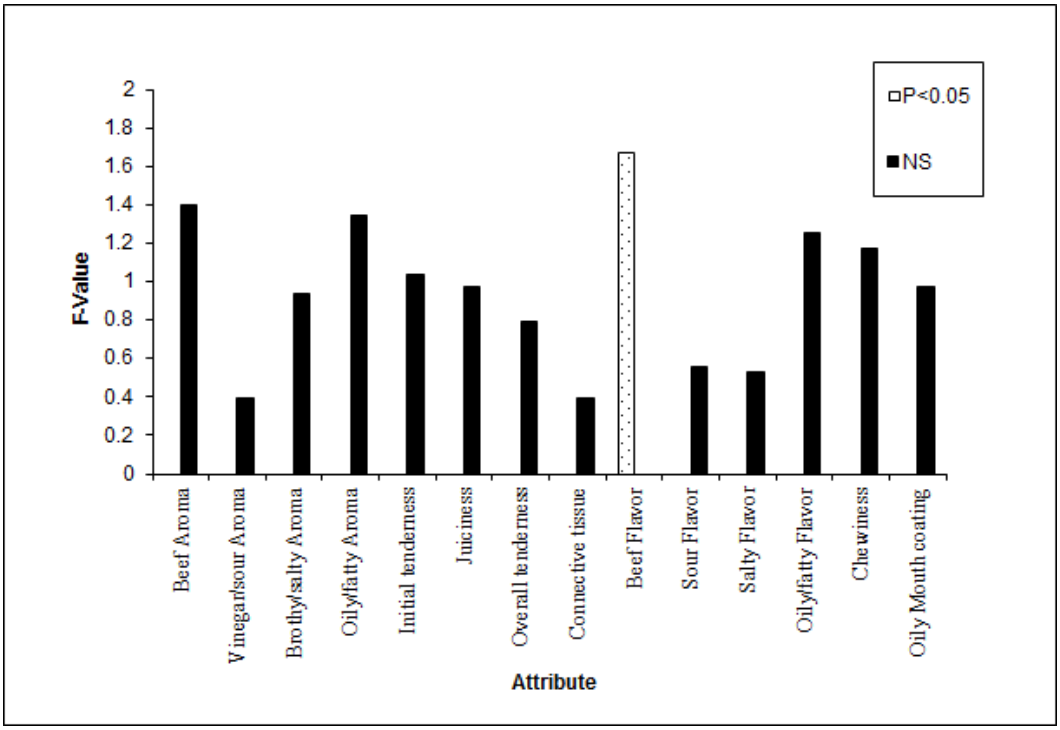

Figure 3.1.5 Panelist/storage day interaction on sensory attributes of bison *LD* muscle.

A relationship between beef aroma and flavour with storage day 21 as well as chewiness and connective tissue with day 2 and 4 were observed in the STATIS PCA bi-plot as shown in Figure 3.1.6. STATIS is a more intuitive and visually powerful analysis method compared to PCA. It is a generalization of principal component analysis (PCA) whose goal is to analyze several sets of variables collected on the same set of observations. The goals of STATIS are 1) to compare and analyze the relationship between the different data sets, 2) to combine them into a common structure called a compromise which is then analyzed via PCA to reveal the common structure between the observations, and finally 3) to project each of the original data sets onto the compromise to analyze commonalities and discrepancies (Abdi & Valentin, 2007). Figure 3.1.6 shows a good separation of 70.3% between storage day 2, 15 and 21 days on the PC1 axis.

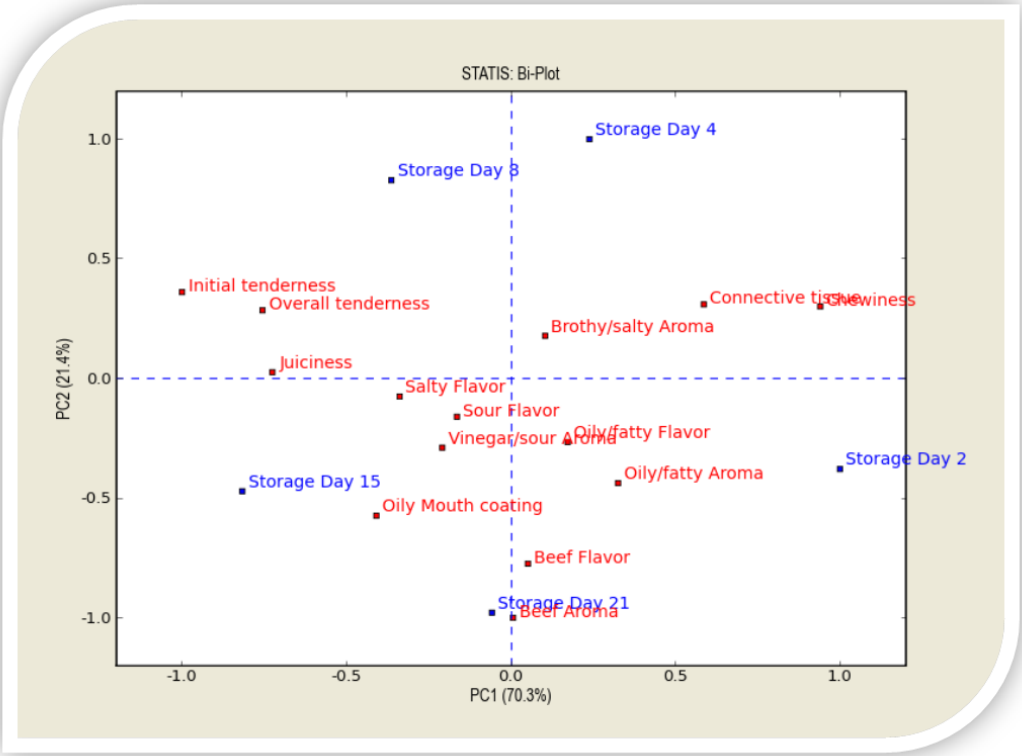

Figure 3.1.6 Relationship between storage day and sensory attributes of bison meat.

3.7 Conclusion

As expected, the *LD* muscle of bison during chilled storage conditioning (4°C) has influenced the sensory qualities of the cooked meat using quantitative descriptive analysis. Of all the attributes of eating quality, tenderness has been rated the most important factor affecting beef palatability (Vasanthi et al., 2007) and has been identified as the most important factor affecting consumer satisfaction and perception of taste (Naveena et al., 2004). Initial and overall tenderness significantly increased ($P<0.05$) in this study from storage day 2 to 15. A correlation with tenderness at storage day 8 and 15 according to the STATIS PCA bi-plot was present in bison. Buffalo meat has been rated superior to beef (Keshava Rao & Kowale, 1986; Valin et al., 1984) while older animals

have more connective tissue and are not preferred due to meat toughness. Approximately 2 year old heifers were used for this study and may be considered more tender than older bisons. Tenderness can also be affected by the cooking method, for example, microwave cooking has been shown to produce less tender (El-Shimi, 1992) and less desirable meat flavours (Hines et al., 1980) while pressure cookers and water baths are cooking methods often used to optimize tenderness due to their rapid cooking and consistent increase of the final internal temperature (Buck et al., 1979). The cooking method applied in this study utilized the natural juices from bison meat while baking in a conventional oven, which may have contributed to the initial and overall tenderness of the meat. Tenderness and juiciness are closely related, the more tender the meat is, the more quickly juices are released by chewing and thus the juicier the meat appears (Cross, 1986). Cooking meat at 100°C converts collagen to gelatin thereby tenderizing the meat (Dransfield, 1983). This temperature can also breakdown fibres in connective tissue which increases tenderness of meat and also reduces chewiness. The mean storage day differences show a significant decrease ($P<0.05$) in connective tissue and chewiness from storage day 2 to 21 in bison samples.

No significant differences ($P<0.05$) between storage days were observed in our study for aroma and flavour other than vinegar/sour aroma. A study by Irueta et al. (2008), observed that there was no significant difference in flavour, odour and amount of connective tissue scores during aging at a significance level of $P<0.05$ in male crossbred water buffaloes of 20 to 24 months old that fed on naturally grown pastures. The study looked at the *LD, biceps femoris (BF), gluteus medius (GM), gastrocgnemius (GG), semimembranosus (SM)* and *semitendinosus (ST)* muscles that were vacuum packed and

stored at 2±1°C for 0, 15 and 25 days while the meat aged and 2.5cm steaks were cooked on an electric grill to an internal temperature of 71±0.5°C. Similar behaviors with flavour and odour were observed along with tenderness, chewiness and juiciness scores (Irurueta et al., 2008) which showed an increment at 15 days of chilled storage conditioning at 4°C in water buffalo meat.

The sensory attribute changes in *post-mortem* bison meat will be further correlated with the chemical analysis of reducing sugars, sugar phosphates, free amino acids, nucleotides, nucleosides, bases and lipids stored at 4°C to compare any flavour trends optimized during cooking in Chapter 5 – Correlation Studies.

Chapter 4 - Raw Meat Quality Analysis

4.1 Sugars, Sugar Phosphates and Sugar Alcohols

Sugars and sugar phosphates are one of the three water-soluble precursors in meat products analyzed in this study. Enzymatic changes in *post-mortem* muscle, affect the concentrations of flavour precursors found in the water-soluble fraction, which includes the breakdown of ribonucleotides to yield free ribose, the increase of free amino acids and peptides through proteolysis and the depletion of glycogen to yield a pool of low molecular weight, sugar-related metabolites (Lawrie & Ledward, 2006). Heating of these products gives rise to meaty aromas (Dwivedi, 1975). The Maillard reaction is a non-enzymatic chemical reaction that occurs between amino acids and reducing sugars and is one of the most important reactions responsible for flavour formation in cooked foods, including meat (Farmer, 1994; Mottram & Nobrega, 1998).

Several sugars and sugar phosphates such as ribose (Aliani & Farmer, 2005b; Mottram & Nobrega, 1998), ribose-5-phosphate (R5P) (Mottram & Nobrega, 1998), glucose, glucose-6-phosphate (G6P) (Farmer et al., 1999), mannose and fructose (Aliani & Farmer, 2005b; Madruga et al., 2010) are usually found in most types of meat such as chicken (Aliani & Farmer, 2005a), beef (Koutsidis et al., 2008a), pork (Meinert et al., 2009) and goat meat (Madruga et al., 2010) and are believed to display flavour-generating potential (Meinert et al., 2009). Ribose, a five carbon sugar and R5P have been shown to be important in aroma development in heated model systems and also contribute to the desirable browning reactions in meat. The addition of small quantities of ribose to raw meat has been shown to increase the quantities of key odour compounds as well as desirable roasty and meaty notes in cooked meat (Farmer et al., 1999). Aliani et

al. (2005) demonstrated the importance of ribose as the key flavour precursor in chicken meat. One study reported that an increase of only two to four fold in ribose concentration (25mg/100g wet weight) was sufficient to significantly increase the desirable aroma and flavour of cooked meat (Aliani & Farmer, 2005a). MacLeod (1994) suggested that in beef, sugars may contribute sweet flavours and aromas upon heating.

Post-mortem conditioning contributed to an increase in ribose concentrations and is likely to have a major effect on flavour formation (Koutsidis et al., 2008a). Ribose significantly increased from conditioning day 1 to day 21 at 4°C in beef *LL* muscle. Ribose has been reported to be the most heat-labile sugar while fructose is the most stable (Macy et al., 1964). Therefore, ribose will likely alter or breakdown during high temperatures. Ribose can be formed from the degradation of R5P (Aliani et al., 2008). R5P has been shown to undergo Maillard reactions significantly faster than similar sugars and sugar phosphates (Sandwick et al., 2005). Madruga et al. (2010) reported that all sugars present in goat meat decreased during cooking, while fructose had the highest percentage loss of 66%. This can be attributed to sugars involvement in the Maillard reaction which produces several pentose and hexose degradation products containing carbonyl groups that are main reactants for the formation of important heterocyclic compounds such as pyrazines, thiazoles and pyridines in meat volatiles (Madruga et al., 2010). The odour obtained from these reactions are dependent on the amino acid, however the nature of the sugar is said to dictate the rate of the reaction (Kiely et al., 1960). In meat, sulfur-containing amino acids particularly cysteine are important for these reactions and will be discussed further in section 4.2.

The present study aimed to investigate the changes in the concentrations of reducing sugars, sugar phosphates and sugar alcohols in bison *LD* muscle during chilled storage conditioning at 4°C.

4.1.1 Materials and Methods

4.1.1.1 Reagents and chemicals

All reference compounds (2-deoxy-D-ribose, D-xylulose-5-phosphate, D-ribulose-5-phosphate, L-rhamnose, D-ribose, ribitol, D-xylose, D-mannose, D-fructose, D-glucose, D-mannitol, myo-inositol, D-ribose-5-phosphate, D-mannose-6-phosphate and D-glucose-6-phosphate) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) and were ≥90% pure. Anhydrous pyridine was also purchased from Sigma-Aldrich Canada Ltd. N,O-bis(trimethylsilyl) trifluoroacetamide (BSFTA) was purchased from Supelco (Bellefonte, PA, USA).

4.1.1.2 Meat samples and extraction method

Meat samples and the water-soluble extraction method used for sugar, sugar phosphate and sugar alcohol analysis are outlined in section 2.2 and 2.4.1, respectively.

4.1.1.3 Silylation of sugars and sugar phosphates

The method used for silylation of sugars and sugar phosphates was adapted from (Aliani et al., 2008). An aliquot of 0.5ml of bison extract containing 300 µl of internal standard rhamnose was frozen at -80°C in 2ml gas-chromatography vials (Fisher Scientific, Ottawa, ON, Canada) and freeze-dried overnight in a Thermo Scientific Heto LyoLab 3000 freeze-drier (Fisher Scientific). Then 120 µl of pyridine and 120 µl of BSTFA were added to freeze-dried samples and briefly vortexed using a Maxi Mix II

(Thermo Scientific, Dubuque, Iowa, USA). Samples were placed in a preheated oven (Lab Line Instrument Incorporated, Melrose Park, IL, USA) at 90°C for 30 minutes and held at room temperature overnight before being transferred to 100 µl glass inserts to be analyzed using gas chromatography-mass spectrometry (GC/MS). Figure 4.1.1 shows ribose plot before and after silylation. Chemical conversion of a compound to an appropriate derivative can sometimes improve the resulting mass-spectral data, either by increasing the compounds vapour pressure or by making its spectrum more easily interpretable (McLafferty & Turecek, 1993).

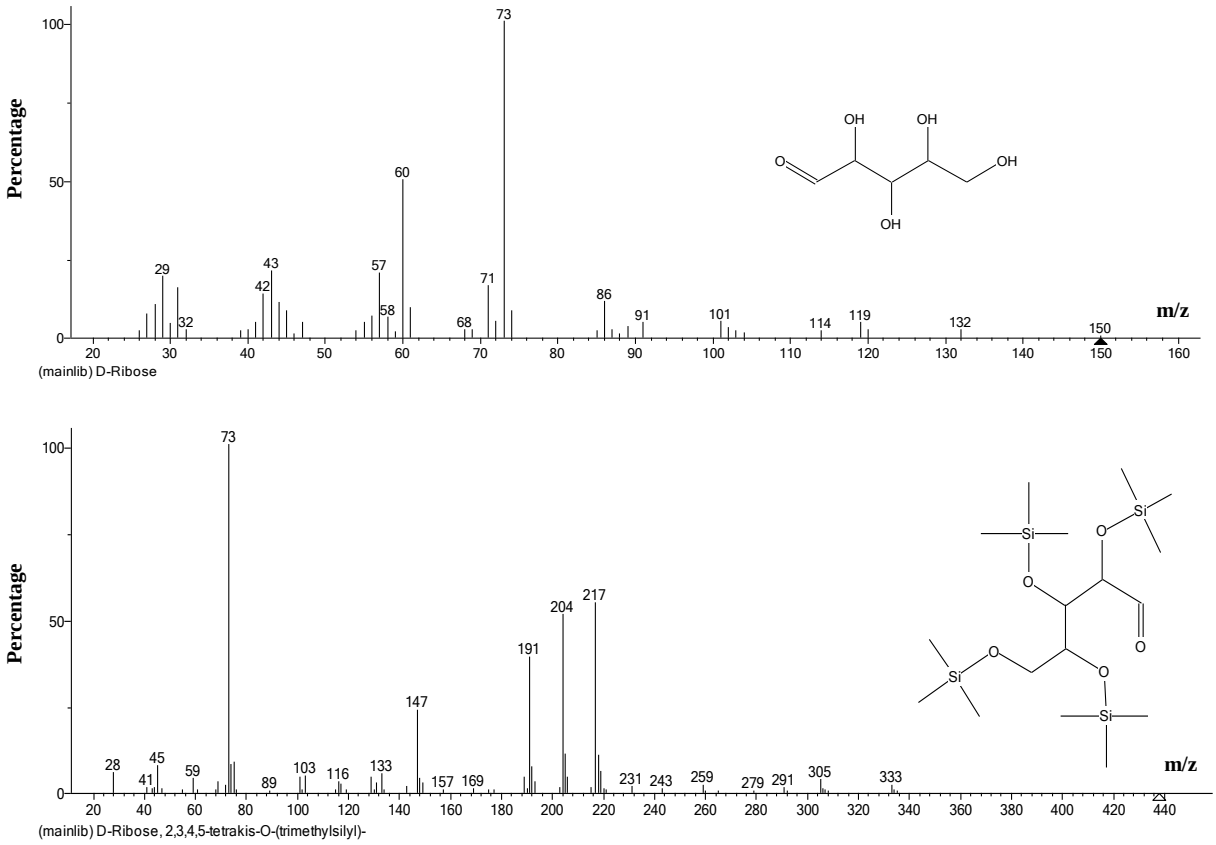

Figure 4.1.1 Ribose before (Top) and after (Bottom) Silylation.

4.1.1.4 Gas chromatography-mass spectrometry

GC-MS analysis of silylated bison water-soluble extracts was performed using a Varian 450GC-240MS/4000 Mass Spectrometry system (Agilent Technologies, Walnut Creek, CA, USA). An aliquot of silylated sugar solution (1.0 µl) was injected at 250°C in split mode (10:1) onto a Factor Four Varian VF-5 30m column (25mm id, 0.25 film thickness). A filament delay of 4 minutes started each run. The oven temperature was initially at 100°C for 1 minute, then increased at 10°C/minute to 230°C and was held for 2 minutes and then increased at 5°C/minute to 250°C and held for 10 minutes. The total run time was 30 minutes. The transfer line was maintained at 270°C. Helium was used as carrier gas with a constant flow rate of 1ml/minute throughout the run. The ion source was operated in full scan electron impact mode (EI) and the mass spectrometer scanned from *m/z* 50 to *m/z* 1000. Hexane was used as a blank and for autosampler wash.

4.1.1.5 Semi-quantification

Semi-quantification of sugars, sugar phosphates and sugar alcohols at different storage conditioning days at 4°C was performed using a suitable internal standard (IS) of rhamnose. Rhamnose is a six carbon sugar and is a naturally occurring deoxy sugar. Rhamnose is often isolated in certain plants and was selected as the IS for sugar and sugar phosphate analysis due to its absence from meat products. The areas of the quantified ions for each sugar, sugar phosphate and augar alcohol was manually integrated in each chromatogram and divided by the area of the quantified ion for rhamnose. Table 4.1.1 lists the quantified ions used for integration of each sugar, sugar phosphate and sugar alcohol. The concentration of rhamnose was divided by the areas to give the concentration for each sugar. Many sugars produced two peaks in each

chromatogram therefore the areas of both peaks were added together in order to see what we would obtain for any concentration in comparison to that of rhamnose.

Table 4.1.1

Quantified ion for sugar, sugar phosphates and sugar alcohols in bison LD muscle extracts.

Sugars, Sugar Phosphates & Sugar Alcohols	**Quantified Ion (Integrated Ion) (m/z)**
Glucose	204
Mannose	204
Xylose	217
Ribose	217
Fructose	437
Mannitol	319
Myo-inositol	305
F6P	589
M6P	387.2
G6P	387.2
Rhamnose (IS)	204

4.1.1.6 Statistical analysis

One-way analysis of variance (ANOVA) was performed using a SPSS (Version 19) software program to analyze sugar and sugar phosphate data. Mean storage day differences for each compound were determined using Tukey's test with a significance level of $P<0.05$.

4.1.2 Results and Discussion

An example of a sugar, sugar phosphate and sugar alcohol chromatogram from a bison extract is shown in Appendix H. The concentration of total reducing sugars, sugar phosphates and sugar alcohols of bison extracts are shown in Table 4.1.2.

Table 4.1.2

Concentration of sugar, sugar phosphate and sugar alcohol products (mmol/kg) in bison LD muscle during post-mortem conditioning over 21 days at 4ºC.

Compounds	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
Glucose	**5.05±1.16[a]**	**4.98±0.88[a]**	**6.86±0.80[b]**	**5.69±0.83[a]**	**5.78±0.61[a]**
Mannose	**0.78±0.46[a]**	**1.08±0.53[ab]**	**1.48±0.60[bc]**	**1.66±0.38[c]**	**1.94±0.48[c]**
Xylose	**0.08±0.0.04[a]**	**0.18±0.05[ab]**	**0.28±0.08[b]**	**0.51±0.16[c]**	**0.62±0.24[c]**
Ribose	**0.06±0.04[a]**	**0.16±0.03[ab]**	**0.39±0.31[bc]**	**0.46±0.10[c]**	**0.58±0.16[c]**
Fructose	**1.32±0.80[a]**	**1.90±0.89[a]**	**2.94±0.87[b]**	**3.32±0.53[b]**	**3.80±0.84[b]**
Mannitol	0.32±0.17	0.35±0.13	0.44±0.11	0.37±0.09	0.36±0.11
Myo-inositol	**1.08±0.74[ab]**	**0.95±0.47[a]**	**1.72±1.04[b]**	**1.32±0.47[ab]**	**1.03±0.33[ab]**
F6P	1.81±0.51	1.88±0.61	2.03±0.65	1.63±0.56	1.53±0.59
M6P	2.51±0.51	2.69±0.45	3.04±0.56	2.60±0.45	2.52±0.69
G6P	**3.27±0.73[a]**	**3.29±0.48[a]**	**4.51±0.61[b]**	**3.81±0.48[ab]**	**3.94±0.80[ab]**

Note. Values are means of 6 animals in duplicate ± standard deviation.

[1]Different letters in the same row indicates a significant difference at $\rho<0.05$.

The total hexose content of glucose, mannose and fructose significantly increased ($P<0.05$) during storage, except that glucose spiked at storage day 8 and decreased thereafter. The expectation is that phosphates of these sugars would breakdown and decrease over extended chilled storage conditioning, however the sugar phosphates remained fairly steady and showed no significant difference ($P<0.05$) between storage days, except for G6P that showed a similar pattern to glucose. The sudden increase of G6P at storage day 8 may be explained by a conversion of glucose-1-phosphate to G6P by *phosphoglucomutase*.

Glucose, G6P and mannose-6-phosphate (M6P) were found to be the major contributors to the sugar and sugar phosphate content in bison extracts with F6P, fructose

and mannose following close thereafter (as shown in Table 4.1.2). Mannose has been reported to have a minor role in relation to flavour generation in pork samples with added mannose (Meinert et al., 2009). Glucose was present in the highest concentration at all five storage conditioning days, with the lowest concentration being 4.98 mmol/kg at storage day 4 to 6.86 mmol/kg at storage day 8. The results for bison *LD* muscle are similar to those reported for sugars in beef (Koutsidis et al., 2008a), pork (Meinert et al., 2009), lamb (Macy et al., 1964) and chicken meat (Aliani & Farmer, 2005b), which have all shown glucose to have the highest concentration, with ribose having the lowest concentration compared to other sugars measured. Pentose sugars, xylose and ribose had the lowest concentrations in bison *LD* muscle. Both sugars increased linearly across storage days, with a significant increase ($P<0.05$) between storage day 2 and 15, with concentrations remaining steady to storage day 21. Bison results are similar to those reported for beef. Koutsidis et al. (2008a) suggested ribose concentrations would increase during chilled storage conditioning. Concentrations of ribose were 0.06, 0.16, 0.39, 0.46 and 0.58 mmol/kg at 2, 4, 8, 15 and 21 storage days, respectively in bison *LD* muscle, while results for beef *LL* muscle were observed at 1, 3, 7, 14 and 21 storage days with concentrations of 0.25, 0.43, 0.76, 1.26 and 1.67 mmol/kg, respectively (Koutsidis et al., 2008a) and are 2 to 4 times higher than results reported in bison. The linear increase in ribose may be a result of the breakdown of inosine-5'-monophosphate (IMP) and the accumulation of its enzymatic breakdown products hypoxanthine, inosine and ribose. This pathway is discussed in further detail in section 4.3. No significant differences ($P<0.05$) between storage days were observed in bison for the sugar alcohol mannitol. Myo-inositol, also a sugar alcohol, showed a random concentration pattern between

storage days with a significant difference (P<0.05) between storage day 4 and 8 as shown in Table 4.1.2.

The total hexose, pentose and sugar phosphate content in bison LD muscle is shown in Figure 4.1.2. No significant differences (P<0.05) were detected in total sugar phosphates. Pentose concentrations may be lower than total hexose and sugar phosphate concentrations however, low concentrations of these pentoses are shown to be major contributors to the formation of flavour of meat.

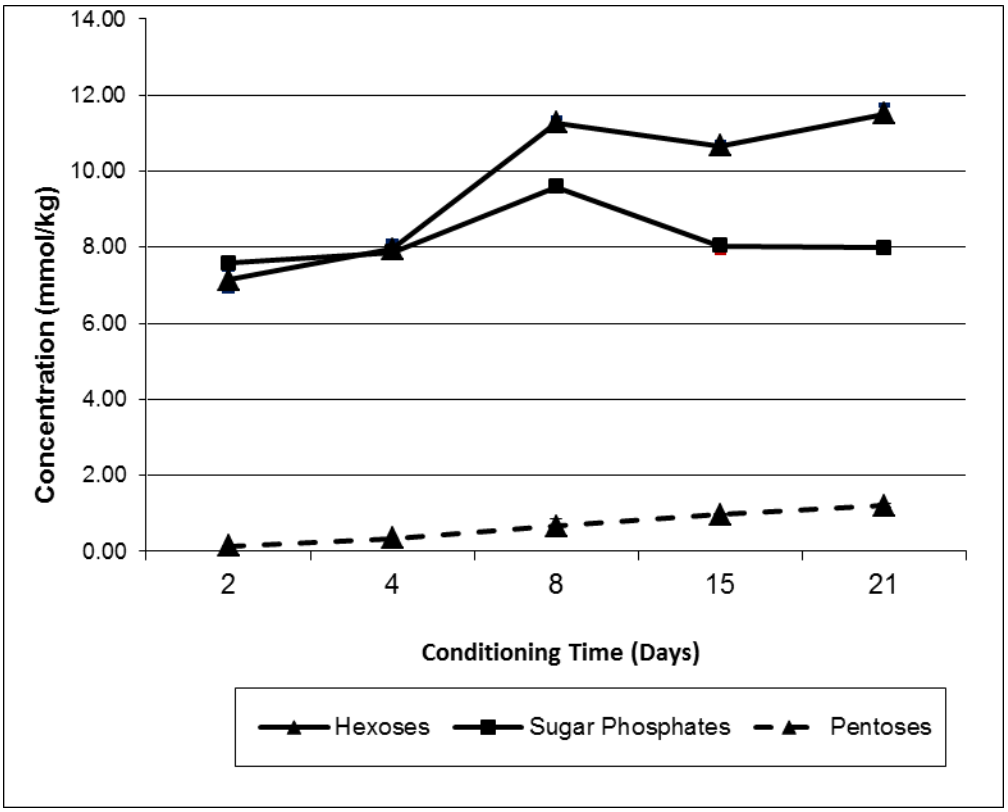

Figure 4.1.2 Total hexose, pentose and sugar phosphate content in bison *LD* muscle during chilled storage conditioning at 4°C. Values are means of 6 animals in duplicate ± standard deviation.

Large standard deviations and coefficients of variations were found in bison *LD* muscle for sugars and sugar phosphates indicating a large animal to animal variation as shown in Table 4.1.2. Large variations were also reported in beef (Koutsidis et al., 2008a), pork (Meinert et al., 2009) and goat meat (Madruga et al., 2010). All six bisons were the same breed and approximate age, fed the same grain-based diet as well as handled and slaughtered under the same conditions; therefore variations are likely a cause of natural genetic differences among animals.

4.1.3 Conclusion

The investigation of the effect of *post-mortem* storage conditioning at 4ºC on concentrations of sugar, sugar phosphate and sugar alcohol flavour precursors of bison *LD* muscle revealed a significant increase of xylose, ribose, mannose and fructose across storage days and a significant spike in glucose, myo-inositol and G6P at storage day 8. These concentration changes can potentially affect the formation of flavour compounds upon heating of meat. No significant differences were observed between storage days for mannitol, F6P and M6P.

As expected, concentrations of ribose significantly increased among storage days. This suggests a possible breakdown of IMP to inosine followed by the accumulation of its enzymatic breakdown products of hypoxanthine and ribose via *inosine nucleosidase*. R5P and ribose-1-phosphate (R1P) were not detected in bison extracts therefore this suggests that either IMP is not broken down to hypoxanthine through a catalyzed reaction by *inosinate nucleosidase* which forms R5P and R1P or due to the potential instability of R1P which caused it to be formed and converted rapidly at earlier stages of conditioning. This therefore suggests, that the AMP pathway might be the major contributor of ribose

in bison extracts over chilled storage conditioning over 21 days at 4°C. Although ribose is present in small concentrations compared to other sugars and sugar phosphates in bison *LD* muscle, it will participate in the formation of potent meat flavour volatiles in model systems upon heating, especially through its contribution to the Maillard reaction.

A discussion of the overall water-soluble (including reducing sugars, sugar phosphates and sugar alcohols) and lipid-soluble results as well as the correlation of reducing sugars and sugar phosphates concentrations to sensory evaluation results will be discussed in Chapter 5 – Correlation Studies.

4.2 Amino Acids

Amino acids are another one of the three water-soluble precursors in meat products analyzed in this study. Many studies have looked at the contribution of amino acids to desirable meaty flavours as a result of the Maillard reaction which produces volatiles during the heating processes of reducing sugars and amino acids , however to the best of our knowledge no studies to date have examined the amino acid content in *Bison bison*. The cooking of meat generates hundreds of volatile compounds, but relatively few make a key contribution to the odour and flavour of cooked meat (Aliani & Farmer, 2005b). Free amino acids have been reported to potentially contribute to sweetness (i.e. alanine and glycine) and bitterness (i.e. leucine, tryptophan, phenylalanine and tyrosine) (Gorbatov & Lyaskovskaya, 1980), while their salts may contribute to a salty and umami taste which is often contributed by glutamic acid and its sodium salt known as monosodium glutamate (MSG) (MacLeod, 1994).

The concentration of free amino acids in meat can be significantly affected by diet and breed (Koutsidis et al., 2008b). Koutsidis et al. (2008b) reported concentrations of glycine, histidine, serine, asparagine, threonine, methionine, tyrosine, phenylalanine, isoleucine, valine, leucine, proline, lysine and tryptophan to be significantly higher ($P<0.05$) in silage versus concentrate fed animals. Lysine (Titgemeyer et al., 1988) and methionine (Fenderson & Bergen, 1975) however have been regarded as the most important limiting amino acids in grain-based diets. Levels of arginine were also reported to be higher in Aberdeen angus beef, while glycine was significantly higher ($P<0.05$) in Hilstein-Friesian steers, which suggests a breed effect on free amino acid content in meat (Koutsidis et al., 2008b). Significant differences were also found in concentrations of free

amino acids between breeds and muscle types of goat (Madruga et al., 2010) and chicken (Aliani & Farmer, 2005a) meat.

Wasserman (1972) emphasized the importance of conditioning in the formation of flavour precursors and suggested that enzymatic changes in muscle can alter the flavour profile of the meat. Enzymatic reactions in *post-mortem* muscle can increase the levels of free amino acids through proteolysis (Wasserman, 1972) which is the degradation of proteins by cellular enzymes called proteases. The rate of pH decline can affect proteolytic activity during early stages of conditioning in *post-mortem* muscle, ultimately influencing the level of free amino acids (O'Halloran et al., 1997). It has been reported that a high pH in *post-mortem* muscle can increase protease activity and further enhance tenderization in the meat (Beltrán et al., 1997).

Sulfur-containing amino acids play a major role in flavour formation of meat. Cysteine is a potent free amino acid precursor of meat flavour volatiles. Heterocyclic compounds such as alkylthiazoles, acylthiazoles, thiols, pyrazines, pyridines and thiophenes have been isolated as volatile products produced by the Strecker degradation of amino acids, especially cysteine (Mottram, 1998). Strecker degradation is a chemical reaction which converts an α-amino acid into an aldehyde by an amine intermediate. Sulfur-containing amino acids such as cysteine are known to be indispensible precursors and their participation in the Maillard reaction and Strecker degradation form characteristic meat odours that breakdown during the cooking of meat (Mottram, 1998) For example, the addition of cysteine to raw muscle favoured the formation of aroma-potent thiophenes in a heated model system (Madruga & Mottram, 1998). Thus, different ratios in amino acid profiles may generate different flavour compounds.

The present study aimed to investigate the changes in the concentrations of the major amino acids in bison *LD* muscle during chilled storage conditioning at 4°C.

4.2.1 Materials and Methods

4.2.1.1 Reagents and chemicals

All reference compounds not included in the EZ:faast for free (physiological) amino acid analysis by GC/MS kit (Phenomenex, Torrance, CA, USA) such as norvaline and L-cysteine were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) and were 99% and 97% pure, respectively. Anhydrous isopropanol and acetone were also purchased from Sigma-Aldrich Canada Ltd. All other reagents and amino acid standard mixtures used for amino acid analysis were included as part of the EZ:faast amino acid kit components.

4.2.1.2 Meat samples and extraction method

Meat samples and the water-soluble extraction method used for amino acid analysis are outlined in section 2.2 and 2.4.1, respectively.

4.2.1.3 Derivatization of amino acids

An aliquot of the centrifuged bison extract (100 µl) contains internal standard norvaline as discussed in section 2.4.1 was derivatized using the EZ:faast amino acid derivatization technique for GC/MS (Phenomenex, Torrance, CA, USA) to determine the free amino acid content of the bison samples. Method sequences for sample preparation by solid phase extraction and derivatization provided in the EZ:faast user manual were closely followed with the exception of the internal standard solution (reagent 1) (0.2mM norvaline and 10% isopropanol) that was replaced with 10% isopropanol for bison

extracts to avoid complications with the addition of extra internal standard. The solid phase extraction was performed via a sorbent tip that binds amino acids while allowing interfering compounds to flow through. Amino acids on sorbent particles were then eluted into the sample vial and quickly derivatized with reagent at room temperature in aqueous solution. Derivatized amino acids then migrated to the organic layer for additional separation from interfering compounds. The organic layer was removed, evaporated, and re-suspended in re-dissolution solvent to be analyzed on the GC/MS. The reason for derivatizing amino acids is the same for silylation of sugars as discussed in section 4.1.1.3.

4.2.1.4 Gas chromatography-mass spectrometry

Analysis of derivatized water-soluble extracts was performed using a Varian 450GC-240MS/4000 Mass Spectrometry system (Agilent Technologies, Walnut Creek, CA). An aliquot of the derivatized amino acid solution (1.5 µl) was injected at 250°C in split mode (20:1) onto a Factor Four Varian VF-5 30m column (25mm id, 0.25 film thickness). A filament delay of 4 minutes started each amino acid run. The oven temperature was initially set at 110°C for 1 minute, then increased at 30°C/minute to 250°C and held for 10.33 minutes. The total run time was 16 minutes. The helium carrier gas flow rate was kept constant throughout the run at 1 ml/minute. The mass spectrometer scanned from *m/z* 50 to *m/z* 550. Isopropanol:acetone (1:1) was used as a blank and for autosampler wash.

4.2.1.5 Semi-quantification

Semi-quantification of amino acids at different storage conditioning days at 4°C was performed using a suitable internal standard (IS) of norvaline. Norvaline is an amino

acid with the formula $C_6H_{11}NO_2$. It was used as the IS for amino acids because it is usually made synthetically and is not found in meat products. The areas of the quantified ions for each amino acid were manually integrated in each chromatogram and divided by the area of the quantified ion for norvaline. Table 4.2.1 lists the quantified ion that was used for integration for each amino acid. The concentration of norvaline of 1.5 mg/ml was then divided by the areas to give the concentration for each amino acid.

Table 4.2.1

Quantified ion for amino acids in bison LD muscle extracts.

Amino Acids	**Quantified Ion (Integrated Ion) (m/z)**
Sarcosine	130
Glycine	74
Valine	116
Leucine	172
Isoleucine	130
Proline	70
Asparagine	69
4-Hydroxy proline	86
Glutamic Acid	84
Phenylalanine	148
α-aminopimelic acid	94
Glutamine	84
Ornithine	70
Lysine	170
Tyrosine	107
Tryptophan	130
Norvaline (IS)	158

4.2.1.6 Statistical analysis

One-way ANOVA was performed using a SPSS (Version 19) software program to analyze amino acid data. Mean storage day differences for each compound were determined using Tukey's test with a significance level of $P<0.05$.

4.2.2 Results and Discussion

The GC/MS analysis of the bison extracts detected several amino acids that may contribute to the flavour of meat upon heating. An example of an amino acid chromatogram from a bison extract is shown in Appendix I. After 21 days of conditioning the total amino acid content of bison *LD* muscle had increased 51% from storage day 4 at 8.65 to 16.95 mmol/kg at storage day 21. A significant increase ($P<0.05$) in total amino acid content is shown in Table 4.2.2. A significant increase ($P<0.05$) is shown from storage day 2 at 9.78 mmol/kg to storage day 15 at 16.95 mmol/kg. The values reported for total amino acid content in bison are similar to the concentrations reported for beef *LL* muscle (Koutsidis et al., 2008a), where it was reported that a 58% increase in total free amino acid content occured from 10.8 mmol/kg at storage day 1 to 18.9 mmol/kg at storage day 21.

The most abundant free amino acids in bison were sarcosine (an intermediate and byproduct in glycine synthesis and degradation), valine, α-aminopimelic acid and glutamine whereas asparagine, 4-hydroxy proline and tryptophan were present in small amounts in bison extracts. Glutamine content in the *LD* muscle of bison had the highest concentration compared to other amino acids which is similar to results reported for pork (Meinert et al., 2009), beef (Koutsidis et al., 2008a) and goat meat (Madruga et al., 2010). The sulfur-containing compounds methionine and cysteine are known to be important flavour precursors, however these were not detected in bison extracts. Cysteine levels are often present in trace amounts in most meat types such as beef (Koutsidis et al., 2008a). Cysteine is unstable and could have either been degraded in the water-soluble extracts or the method used for this study was not sensitive enough for cysteine analysis.

No significant differences ($P<0.05$) between conditioning days were observed in proline, α-aminopimelic acid, glutamine and ornithine content as shown in Table 4.2.2. The concentrations of all the other amino acids increased significantly ($P<0.05$) over chilled storage conditioning for 21 days at 4ºC. Valine, leucine and isoleucine all showed a significant increase from storage day 4 to 21, whereas asparagine, 4-hydroxy proline, lysine and tyrosine showed a significant increase between storage day 2 and 15 with concentrations remaining constant thereafter. Sarcosine, glycine, glutamic acid and tryptophan did not show any significant concentration differences ($P<0.05$) until approximately day 8 to 15 in the conditioning process. Concentrations of phenylalanine increased significantly ($P<0.05$) from 0.21 to 0.93 mmol/kg at storage day 2 to 21, respectively. Valine, leucine, isoleucine and phenylalanine are important amino acids in flavour formation, providing Strecker aldehydes, such as 2- and 3-methylbutanals and other aroma compounds such as pyrazines (Koutsidis et al., 2008a).

Table 4.2.2

Concentrations of free amino acids (mmol/kg) in bison LD muscle during post-mortem conditioning over 21 days at 4ºC.

Compounds	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
Sarcosine	**1.01±0.31[a]**	**0.87±0.46[a]**	**1.21±0.16[ab]**	**1.45±0.29[b]**	**1.45±0.51[b]**
Glycine	**0.29±0.10[a]**	**0.29±0.11[a]**	**0.36±0.08[a]**	**0.49±0.08[b]**	**0.53±0.08[b]**
Valine	**0.75±0.17[a]**	**0.84±0.28[a]**	**1.39±0.20[b]**	**2.25±0.37[c]**	**2.95±0.81[d]**
Leucine	**0.29±0.10[a]**	**0.37±0.09[a]**	**0.60±0.04[b]**	**0.95±0.11[c]**	**1.19±0.28[d]**
Isoleucine	**0.32±0.10[a]**	**0.43±0.11[a]**	**0.71±0.05[b]**	**1.15±0.09[c]**	**1.48±0.31[d]**
Proline	0.88±1.06	0.53±0.11	0.69±0.09	0.88±0.13	1.00±0.19
Asparagine	**0.13±0.06[a]**	**0.14±0.05[a]**	**0.21±0.04[b]**	**0.31±0.05[c]**	**0.34±0.08[c]**
4-Hydroxy proline	**0.10±0.04[a]**	**0.10±0.02[a]**	**0.18±0.03[b]**	**0.28±0.07[c]**	**0.33±0.07[c]**
Glutamic Acid	**0.31±0.19[a]**	**0.30±0.10[a]**	**0.38±0.04[ab]**	**0.46±0.06[bc]**	**0.54±0.08[c]**
Phenylalanine	**0.21±0.07[a]**	**0.32±0.07[b]**	**0.54±0.04[c]**	**0.77±0.07[d]**	**0.93±0.16[e]**
α-aminopimelic acid	1.35±0.46	1.28±0.48	0.98±0.36	1.12±0.46	1.20±0.53
Glutamine	3.25±1.58	2.12±1.01	2.92±1.14	3.13±1.60	2.23±1.39
Ornithine	0.22±0.17	0.19±0.15	0.25±0.24	0.25±0.19	0.20±0.13
Lysine	**0.31±0.10[a]**	**0.38±0.08[a]**	**0.61±0.06[b]**	**0.97±0.23[c]**	**1.12±0.32[c]**
Tyrosine	**0.26±0.06[a]**	**0.36±0.04[a]**	**0.72±0.15[b]**	**1.07±0.24[c]**	**1.23±0.34[c]**
Tryptophan	**0.09±0.03[a]**	**0.11±0.04[ab]**	**0.10±0.08[a]**	**0.19±0.12[bc]**	**0.22±0.10[c]**
Total amino acids	**9.78±2.62[a]**	**8.65±2.69[a]**	**11.85±1.68[ab]**	**15.72±2.58[bc]**	**16.95±2.11[c]**

Note. Values are means of 6 replicates in duplicate ± standard deviation.

[1]Different letters in the same row indicates a significant difference at $\rho<0.05$.

4.2.3 Conclusion

Amino acids are important for the formation of aroma volatiles produced by the Maillard reaction and Strecker degradation, while the amino acid profiles result in the generation of different flavour compounds (Kiely et al., 1960). The effect of *post-mortem* conditioning at 4ºC on concentrations of amino acid water-soluble flavour precursors of

the *LD* muscle revealed a significant increase in all amino acids with the exception of proline, α-aminopimelic acid, glutamine, and ornithine. The concentration changes in amino acids can potentially affect the formation of flavour compounds upon heating. The sulfur-containing amino acids cysteine and methionine that produce volatile sulfur-containing compounds were not detected in bison extracts. As mentioned previously, this may be due to cysteine's quick degradation or the method used is not sensitive enough to detect cysteine. The method used in this study has been used in other studies such as beef (Koutsidis et al., 2008a) and have used the same derivatization method to analyze cysteine; however cysteine was only detectable in trace amounts. Cysteine is considered to be important due to its reaction with ribose when meat is cooked. The aromatic compounds phenylalanine and tyrosine were present in adequate amounts in bison extracts.

The essential amino acids leucine, phenylalanine, tryptophan and tyrosine are known to produce a bitter taste, while isoleucine, lysine, valine and the nonessential amino acid proline are tasteless (Gorbatov & Lyaskovskaya, 1980). Glycine may contribute to the sweet taste of meat (Gorbatov & Lyaskovskaya, 1980). These compounds are all present in bison extracts and in combination with each other may produce a desirable amino acid flavour profile. Other amino acids not detected in bison extracts such as alanine, cysteine, methionine and arginine may contribute a sweet, sulphurous, meaty and tasteless attributes, respectively. Regardless of the concentration or individual effect on taste of each amino acid, these precursors in combination upon heating contribute to the overall flavour of the meat.

A discussion of the overall water-soluble (including amino acids) and lipid soluble results as well as the correlation of amino acid concentrations to sensory evaluation results will be discussed in Chapter 5 – Correlation Studies.

4.3 Nucleotides, Nucleosides and Bases

Nucleotides, nucleosides and bases are the third group of water-soluble flavour precursors in meat products analyzed in this study. Studies on 5'-mononucleotides have been evaluated in relation to their ability to intensify or modify the flavour of foods (Gorbatov & Lyaskovskaya, 1980). Ribonucleotides, such as IMP and guanosine-5'-monophosphate (GMP), play important roles in the flavour of meat and help suppress sulfury, fatty, starchy, bitter and hydrolyzed vegetable type flavours in meat while enhancing meaty flavours (Arya & Parihar, 1979). Hypoxanthine, IMP and GMP contribute an umami taste, which is described as a pleasant brothy or meaty taste which is intensified when ribonucleotides are combine with glutamate, however hypoxanthine may also contribute to bitterness (MacLeod, 1994).

IMP breaks down upon heating and yields ribose and ribose phosphates (Madruga, 1994). Pentoses, particularly ribose derived from ribonucleotides such as inosine are important precursors for chemical reactions in meat (Mottram, 1998). In muscle, ATP is essential for muscle function and is converted to IMP post-slaughter (Mottram, 1998). Initial ATP levels are variable and dependent on species, type of muscle and pre-slaughter conditions (Lawrie, 1998). The generally accepted route for the formation of ribose in meat is via the breakdown of ATP to adenosine 5'-monophosphate (AMP) and then from IMP to inosine and then to ribose and hypoxanthine as shown by the **bold** arrows in Figure 4.3.1 (Aliani et al., 2008). This pathway seems to be occurring in most types of meat however, in most reported studies, only nucleotides, nucleosides and bases were determined and no information with regards to ribose or other sugars was provided. Therefore, the information provided for hypoxanthine may not necessarily be

related to above reaction as it is important to note that other biochemical routes may also promote the formation of hypoxanthine. For instance, the breakdown of inosine to R1P and hypoxanthine by *purine-nucleoside phosphorylase* might partly explain the formation of hypoxanthine in meat while IMP can also form R5P and hypoxanthine through a reaction catalyzed by *inosinate nucleosidase*. Previous studies on chicken crude extract demonstrated, that even in the presence of inhibitors of *inosine nucleosidase*, the enzyme catalyzing the breakdown of inosine (inosine → hypoxanthine and ribose), hypoxanthine was formed, indicating that other pathways are also involved (Aliani et al., 2008). In some species, such as marine invertebrate muscle from Mollusca, an active *adenosine aminohydrolase* facilitates the conversion of AMP to inosine via adenosine rather than IMP (Hiltz & Bishop, 1975). Therefore, the hypoxanthine content in meat may not be from a single metabolic pathway. The structure of IMP, inosine and hypoxanthine are shown in Figure 4.3.2.

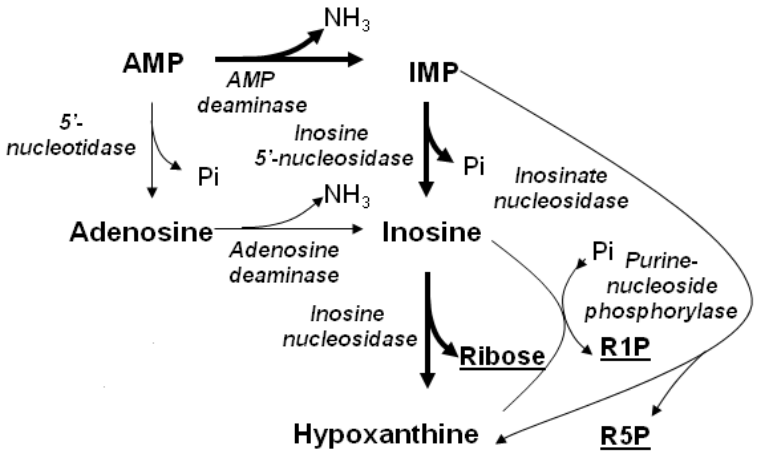

Figure 4.3.1 Breakdown pathways that might occur in chicken (Aliani et al., 2008).

Changes in these nucleotide, nucleoside and base compounds have been followed during the aging of meat from pigs (Gorbatov & Lyaskovskaya, 1980), beef (Koutsidis et al, 2008a) and chicken (Aliani & Farmer, 2005a). Kinsmen et al. (1994) suggested that inosine and hypoxanthine concentrations increase with prolonged storage once AMP has entered a catabolic pathway. Concentrations of inosine and hypoxanthine can also be used as an indicator of freshness in muscle foods (Kinsmen et al., 1994).

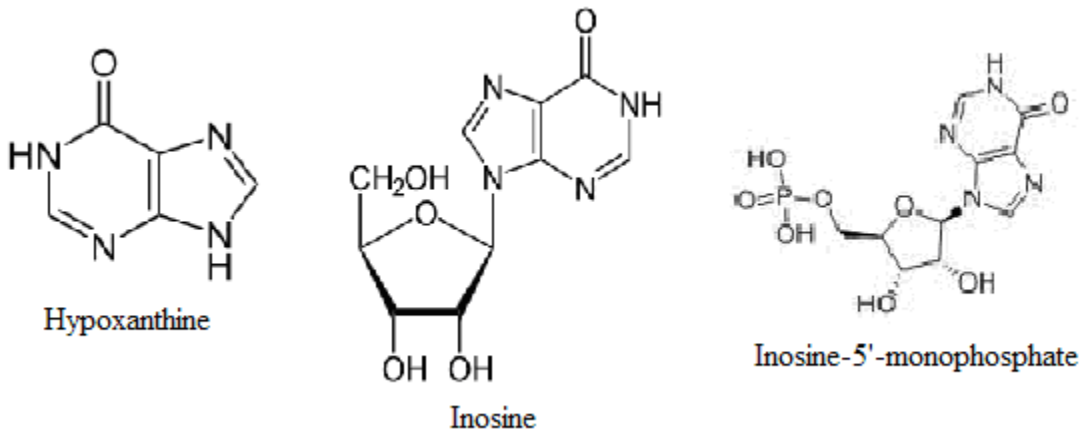

Figure 4.3.2 Structures of hypoxanthine, inosine and IMP.

This present study aimed to investigate the concentration changes of IMP, inosine and hypoxanthine in bison *LD* muscle during chilled storage conditioning at 4°C.

4.3.1 Materials and Methods

4.3.1.1 Reagents and chemicals

All reference compounds (creatinine, cytidine, ATP, IMP, hypoxanthine and inosine) were purchased from Sigma-Aldrich (Oakville, ON, Canada) and were ≥98%

pure. Potassium phosphate monobasic (KH_2PO_4) and hydrochloric acid were also purchased from Sigma-Aldrich Canada Ltd.

4.3.1.2 Meat samples and extraction method

Meat samples and the water-soluble extraction method used for nucleotide, nucleoside and base analysis are outlined in sections 2.2 and 2.4.1, respectively.

4.3.1.3 High pressure liquid chromatography

A Varian 920 high pressure liquid chromatography (HPLC) instrument with a 4.6 X 250mm Sheresorb ODS2 C18 column, 5µm particle size from Waters Incorporated (Mississauga, ON, Canada) was adapted from Aliani et al. (2008) and was used for nucleotide, nucleoside and base analysis. An injection of bison sample extract (20 µl) as outlined in section 2.4.1 was eluted with an isocratic mobile phase KH_2PO_4 (50mM) prepared with HPLC grade distilled water, pH was adjusted to 2.5 with the addition of monobasic anhydrous hydrochloric acid (approximately 2 to 4ml added) and filtered using 0.22µm MAGNA nylon membrane filters (GE Water and Process Technologies, Oakville, ON, Canada) and an electric vacuum pump prior to use. An Accumet Basic AB15 Plus pH meter (Fisher Scientific, Ottawa, ON, Canada) was used to monitor the final pH values. Fresh buffer was prepared and changed after 30 runs. Preconditioning of the HPLC column consisted of a 5 minute elution with distilled water followed by 5 minutes with buffer. An isocratic method was used with a flow rate of 1 ml/minute from 0 to 5 minutes which was then adjusted to 0.5 ml/minute for 1 minute (from 5 to 6 minutes) and was held at this flow rate for 4 minutes. A pump gradient between 10 to 12 minutes increased to a flow rate of 1.5 ml/minute and was held for 6 minutes. The pressure gradient then decreased to a flow rate of 1 ml/minute between 18 and 20 minutes

and held constant and stable for 2 minutes. The total run time was 22 minutes with UV detection at 254 nm. All samples were extracted and analyzed in duplicate.

4.3.1.4 Calibration curves

The calibration curves were prepared using a range of standards containing various amounts of nucleotides, nucleosides and bases. Standard solutions containing a mixture of ribonucleotides were prepared in 5 ml of distilled water and contained 0.29 to 2.19 mg IMP, 0.02 to 0.68 mg hypoxanthine and 0.07 to 0.84 mg inosine. Cytidine was used as the internal standard (IS) and 0.20 mg was added to each solution and mimics the amount added to bison extracts. Standards were eluted with 50mM potassium phosphate buffer and 20 µl of each solution was injected using the method described in the previous section. The reference peak area (RPA) was plotted against the quantity of each nucleotide, nucleoside and base. Calibration curves and linear regression equations for IMP, hypoxanthine and inosine are shown in Figure 4.3.3, 4.3.4 and 4.3.5, respectively. The curve and linear regression equation were used to calculate the concentrations of nucleotides, nucleosides and bases within bison extracts.

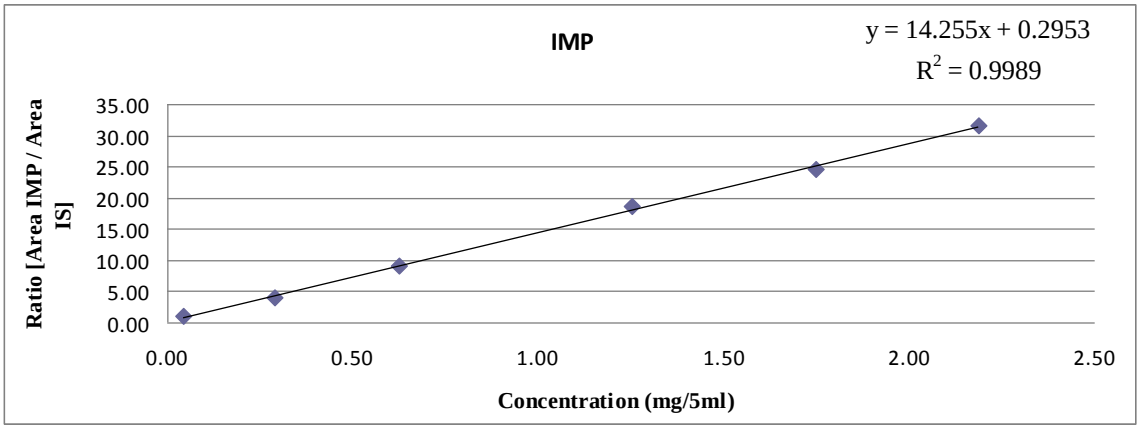

Figure 4.3.3 Calibration curve based on different concentrations of IMP.

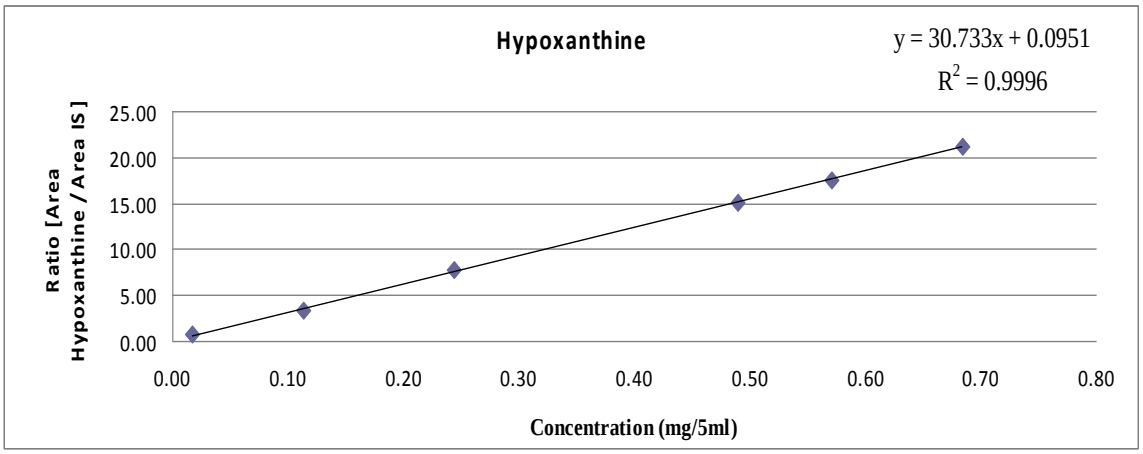

Figure 4.3.4 Calibration curve based on different concentrations of hypoxanthine.

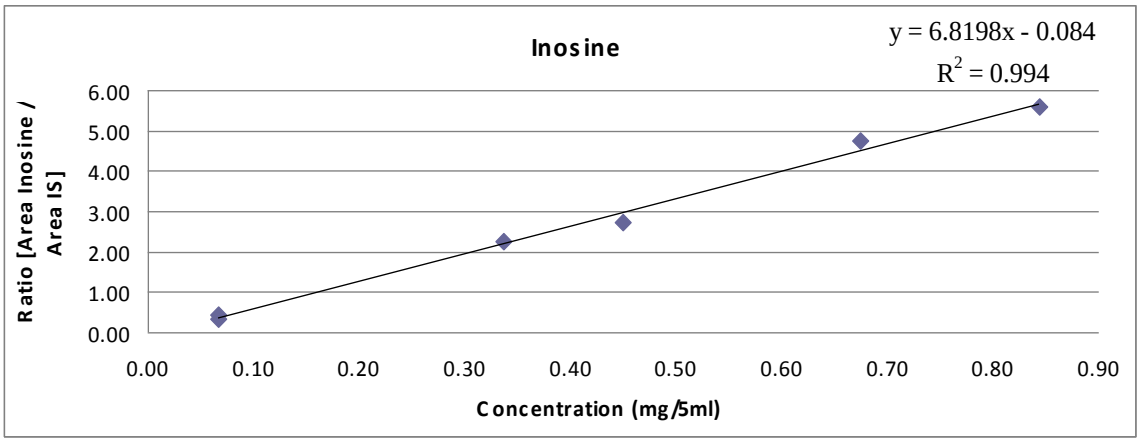

Figure 4.3.5 Calibration curve based on different concentrations of inosine.

4.3.1.5 Statistical analysis

One-way ANOVA was performed using a SPSS (Version 19) software program to analyze nucleotide, nucleoside and base data. Mean storage day differences for each compound were determined using Tukey's test with a significance level of $P<0.05$.

4.3.2 Results and Discussion

The degradation of IMP and the accumulation of its enzymatic products, hypoxanthine and inosine, are evident in bison extract chromatograms. An example of a

nucleotide, nucleoside and base chromatogram from a bison extract and a standard mixture are shown in Appendix J and K. The degradation of IMP did not occur rapidly between day 2 and 8, however a significant decrease ($P<0.05$) is shown between storage day 2 and 15 from an average of 6.55±1.46 to 2.92±0.91 mmol/kg and remained stable to day 21 at 1.99±0.60 mmol/kg (see Table 4.3.1). At the end of 21 days of conditioning there was still sufficient IMP to be degraded further into inosine, hypoxanthine and ribose. As shown in Table 4.3.1, there was a gradual increase in inosine with a significant difference from day 2 to 15 days of conditioning and this remained constant until day 21. While inosine remained unchanged after 15 days of conditioning, hypoxanthine continued to significantly increase from 1.29±0.51 at day 2 to 4.40±0.99 mmol/kg at day 21 as shown in Figure 4.3.6. This could be an indication that IMP, rather than being directly dephosphorylated to inosine, is possibly hydrolyzed to hypoxanthine and R5P (Koutsidis et al., 2008a), however R5P was not detected in bison extracts.

Table 4.3.1

Concentration of nucleotide degradation products (mmol/kg) in bison LD muscle during post-mortem conditioning over 21 days at 4°C.

Compound	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
IMP	**6.55±1.46[a]**	**6.50±1.67[a]**	**5.29±2.07[a]**	**2.92±0.91[b]**	**1.99±0.60[b]**
Inosine	**0.09±0.03[a]**	**0.13±0.03[b]**	**0.16±0.03[b]**	**0.21±0.02[c]**	**0.23±0.03[c]**
Hypoxanthine	**1.29±0.51[a]**	**1.79±0.51[ab]**	**2.74±0.47[bc]**	**3.59±1.30[cd]**	**4.40±0.99[d]**
Total Nucleotides	**7.93±1.18[ab]**	**8.41±1.17[a]**	**8.18±2.16[ab]**	**6.71±1.72[ab]**	**6.61±0.98[b]**

Note. Values are means of 6 animals in duplicate ± standard deviations.

[1] Different letters in the same row indicates a significant difference at $\rho<0.05$.

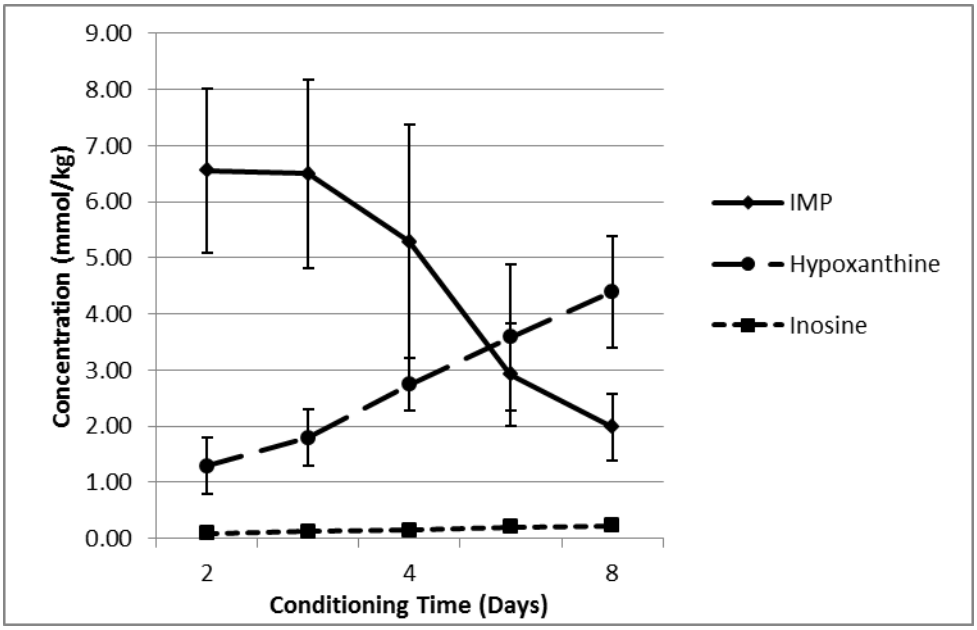

Figure 4.3.6 Mean concentration changes of IMP, inosine and hypoxanthine in bison *LD* muscle during chilled storage conditioning at 4°C. Values are means of 6 animals in duplicate ± standard deviation.

Figures 4.3.7, 4.3.8 and 4.3.9 compare the mean concentration changes of IMP, inosine and hypoxanthine during conditioning at 4°C, over 21 days for bison (*LD)*, beef *(LL)* (Koutsidis et al., 2008a), chicken breasts (Aliani & Farmer, 2005a) and goat meat (Madruga et al., 2010). Koutsidis et al. (2008) reported similar results for IMP in beef with 6.27±0.17 to 2.63±0.12 mmol/kg from 1 to 21 days, respectively. A similar pattern with hypoxanthine and inosine in the *LL* muscle of beef during *post-mortem* conditioning at 4°C was also observed (Koutsidis et al., 2008a).

Certain degradation products such as creatine, creatinine and AMP could not be detected with the HPLC method used or were present in trace amounts in meat extract.

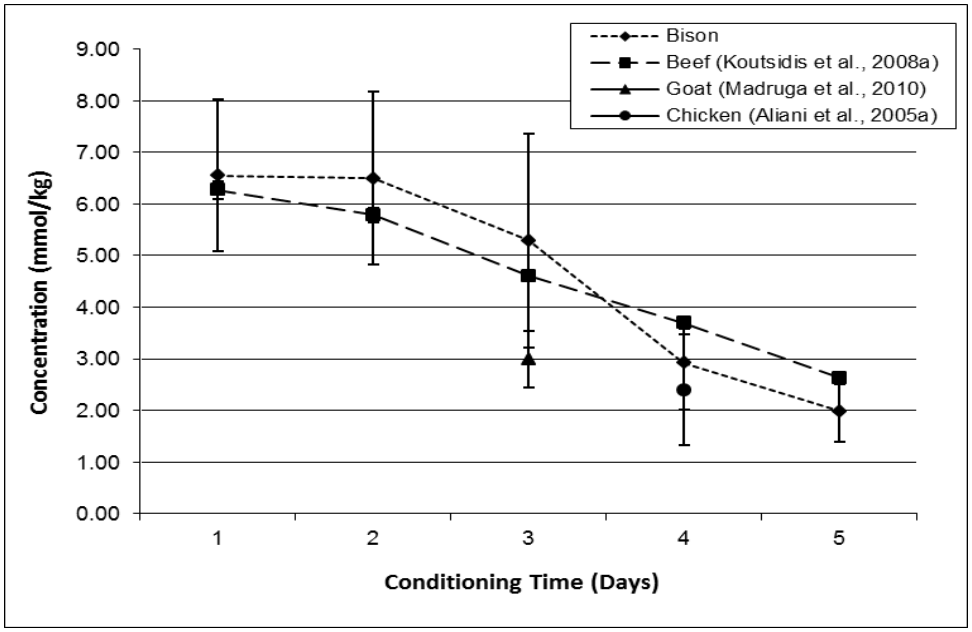

Figure 4.3.7 Comparing IMP mean concentrations of bison (*LD*), beef (*LL*), goat and chicken (breast) meat during chilled storage conditioning for 21 days at 4°C. Conditioning days represent day 2, 4, 8, 15 and 21 days for bison; day 1, 3, 7, 14 and 21 days for beef, day 7 for goat and day 14 for chicken.

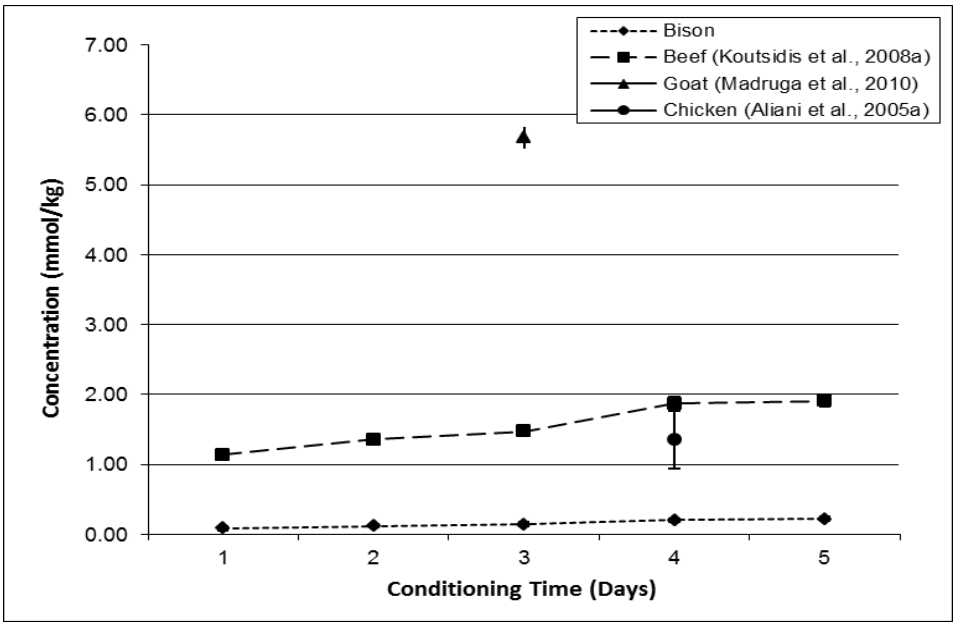

Figure 4.3.8 Comparing inosine mean concentrations of bison (*LD*), beef (*LL*), goat and chicken (breast) meat during chilled storage conditioning for 21 days at 4°C. Same conditioning days as shown in Figure 4.3.7.

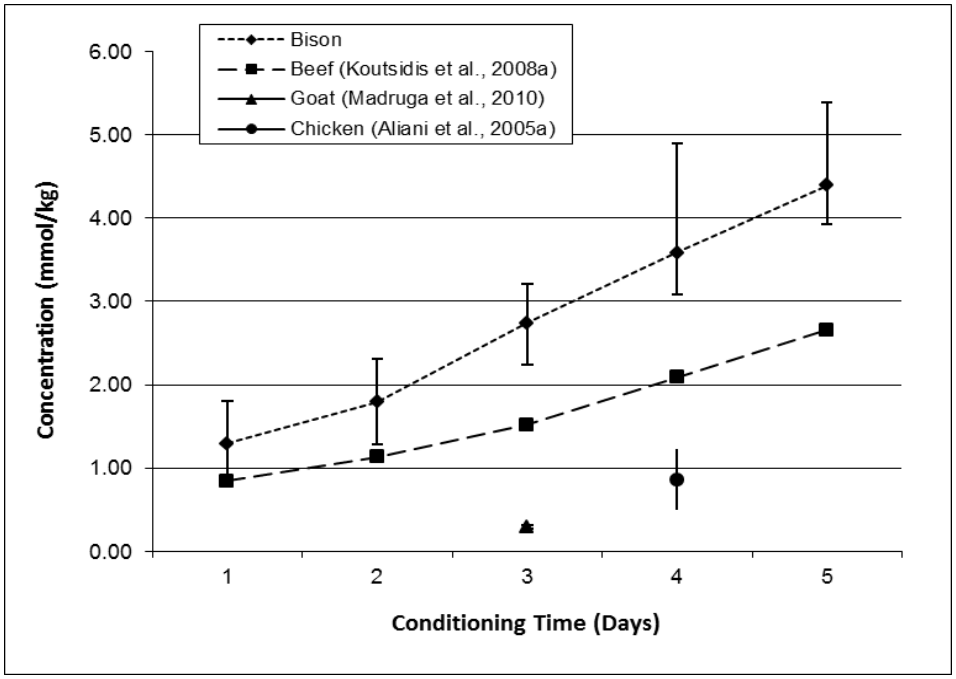

Figure 4.3.9 Comparing hypoxanthine mean concentrations of bison (*LD*), beef (*LL*), goat and chicken (breast) meat during chilled storage conditioning for 21 days at 4°C. Same conditioning days as shown in Figure 4.3.7.

4.3.3 Conclusion

The investigation of the effect of *post-mortem* storage conditioning at 4°C on concentrations of nucleotide, nucleoside and base water-soluble flavour precursors of bison *LD* muscle revealed a significant decrease of IMP and an increase of inosine and hypoxanthine across storage days. These concentration changes can potentially affect the formation of flavour compounds upon heating of meat. Concentrations of AMP were non-existent suggesting that by 2 days *post-mortem* AMP has been converted to IMP via the enzyme *AMP deaminase.* As expected, conditioning decreased concentrations of IMP and as a result concentrations of inosine via the enzyme *inosine-5'-nucleosidase* and hypoxanthine via *inosine nucleosidase* increased due to degradation processes of IMP in

bison meat. Results do not suggest that the direct conversion of AMP to inosine took place through the conversion of adenosine since traces of adenosine were not evident in bison extracts.

Creatine and creatinine have been reported to be associated with the formation of heterocyclic model systems in cooked meat (Jägerstad et al., 1991). The normal transformation of creatine and creatinine post-slaughter could not be identified in bison extracts. Creatine can be converted non-enzymatically to creatinine both *in vivo* (Wyss & Kaddurah-Daouk, 2000) and *in vitro* during heating of creatine (Macy et al., 1970) and should be considered as a compound of interest for future flavour research of bison meat.

A discussion of the overall water-soluble (including nucleotides, nucleosides and bases) and lipid-soluble results as well as the correlation of nucleotide, nucleoside and base concentrations to sensory evaluation results will be discussed in Chapter 5 – Correlation Studies.

4.4 Lipids

The second category of meat flavour precursors in addition to water-soluble components are lipids. Lipids are present in muscles as structural components to muscle membrane, as storage droplets for triacylglycerol between muscle fibres as well as adipose tissue also known as the marbling fat (Enser, 2001). In *post-mortem* muscle, lipids not only serve as an energy source stored in animal muscle but also contribute to the flavour of meat and its quality attributes, particularly their fatty acids. While water is the most prevalent component of meat at approximately 73% (Lambert et al., 1991), most of the flavour and aroma-carrying molecules are *hydrophobic* and are therefore dissolved in fat (Enser, 2001). The four naturally occurring molecules of focus for this research were (a) free fatty acids which are carboxylic acids with a long unbranched aliphatic chain that can be saturated or unsaturated, (b) triglycerides which are esters derived from glycerol and three fatty acids, (c) phospholipids which are major components of all cell membrane as they form phospholipid bilayers, most of which contain a diglyceride, a phosphate group and a simple organic molecule and (d) cholesterol which is a waxy steroid of fat manufactured in the liver or intestines. Fatty acids are often derived from triglycerides and phospholipids.

In fresh meat, lipids contribute to the overall colour, drip loss and degree of oxidative rancidity (Enser, 2001). The flavour of meat can often be attributed to the marbling fat which can also contribute to the overall juiciness of the meat, however this is dependent on the thermal oxidation of the lipids (Enser, 2001), lipid oxidation and/or degradation. Marbling fat has been reported to have more predictable effects on juiciness and flavour over tenderness of meat (Thu, 2006). It is also responsible for flavour

differences among species. Lipids may breakdown via fatty acid oxidation and give rise to volatile odour compounds which contribute to both desirable and undesirable flavours (Mottram, 1987). Many studies have reported several hundred volatile compounds from fatty acid oxidation during lipid degradation in meat such as aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids and esters (Mottram, 1998). It has also been reported that during long-term storage, these reactions may lead to rancid off-flavours, however in cooked meat these reactions occur rapidly and contribute to desirable flavours (Mottram, 1998).

The variation in lipid content may also result in different flavours. Polyunsaturated (PUFA) are more susceptible to oxidative breakdown during cooking than monounsaturated (MUFA) or saturated (SAT) fatty acids leading to a greater quantity of volatile products formed (Thu, 2006). Phospholipids contain higher proportions of unsaturated fatty acids, particularly arachidonic acid (20:4), which undergoes oxidation more readily than saturated fatty acids, making them an important source of volatiles during cooking (Mottram, 1998) and which contribute to the formation of desirable meat flavours (Farmer, 1994). Phospholipids are reported to contribute to the aroma development in meat whereas triglycerides appear not to be as important (Mottram, 1996). Reports suggest that lipid oxidation and the Maillard reaction do not occur in isolation but rather interact together and produce a wide range of effects on the volatile aroma compounds produced (Farmer et al., 1989), by reducing potent odours from certain products of both reactions. The odour threshold from lipid-derived compounds are generally higher than water-soluble precursors such as sulfur and nitrogen heterocyclic compounds (Mottram, 1998). Evidence also suggests that lipids appear to

control the production of sulfur compounds during cooking of meat and suggests that these compounds are therefore maintained at optimum levels in the cooked meat (Mottram, 1998). Saturated and unsaturated aldehydes from lipid autoxidation are contributors in the volatile profile of cooked meats, particularly through reactions between carbonyl compounds and amino as well as thiol groups through the Maillard reaction (Mottram, 1998).

The nutrient composition of bison and other meats are dependent on a number of factors including age, type of feed, gender, cut of meat. The fat content of meat varies from animal to animal and part to part. Different flavour profiles are generated from fatty tissues and are different among animal types such as pork, beef, chicken, turkey and lamb (Thu, 2006). The animal's age also plays a role in how much fat is in the meat. In general, the older the animal, the more time it has had to build fat reserves in its muscles. The older animal will produce greater flavours derived from lipids (Thu, 2006). Diet can also play a role in the fatty acid composition of meat. Larick and Turner (1990), found differences in the PL fatty acids between grass and grain finished steers which resulted in different types of flavour volatiles. Differences occurred in grass-finished animals which contained higher levels of α-linolenic acid (C18:3n3) and n-3 PUFAs synthesized from it, while the grain-finished animals contained higher levels of linoleic acid (C18:2) and other n-6 PUFAs (Larick & Turner, 1990). Reports have shown that if linoleic acid in sheep and cattle adipose tissues is high, it will produce oily, sweet or bland tastes during cooking, however high concentrations of oleic acid will improve the flavour of the meat (Thu, 2006). Appropriate temperature holding and packing of meat can also contribute to meat flavour, particularly if high concentrations of linoleic acid are present since it can

easily combine with atmospheric oxygen to give rise to oxidative rancidity (Thu, 2006). Vacuum packaging was used in this study to prevent oxygen from interfering with molecules and further increase the rate of spoilage.

In addition to flavour, we must not ignore that the mainstream medical and news-media have long advised that the quantity and types of fats consumed play a role in cardiovascular disease risk and health status (Health Canada, 2008). At the forefront of choosing alternative meats, bison has been deemed to have fewer calories and lower fat content (Driskell et al., 1997; McClenahan & Driskell, 2002) than beef. Fat is an important part of a healthy diet because it provides essential fatty acids and energy. Fat also serves as a carrier for the absorption of vitamin A, D, K, E and carotenoids (Health Canada, 2008; USDA, 2005). The USDA (2005) recommended a total daily intake of fat between 20 to 35% of calories as well as a daily cholesterol intake of less than 300mg/day. Cholesterol can increase the risk of unhealthy blood lipid levels, which may increase the risk of coronary heart disease (USDA, 2005).

The present study aimed to investigate the changes in the percentages of fatty acids in free fatty acids, triglycerides and phospholipids as well as concentrations of cholesterol in bison *LD* muscle during chilled storage conditioning at 4°C.

4.4.1 Materials and Methods

4.4.1.1 Reagents and chemicals

Chloroform (≥99%), methanol (≥99%), anhydrous isopropanol (99.8%), 0.5N methanolic KOH, 8-anilino-1-naphthalene sulfonic acid (ANSA), hexane, petroleum ether, anhydrous diethyl ether, acetic acid and BF_3 methanol chemicals and compounds

used for lipid analysis were all purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

4.4.1.2 Meat samples and extraction method

Meat samples and the lipid-soluble extraction method used for FFA, TG, PL and cholesterol analysis are outlined in sections 2.2 and 2.4.2, respectively.

4.4.1.3 Cholesterol analysis

Cholesterol was analyzed using a cholesterol-SL assay kit procedure (Diagnostic Chemicals Ltd., Charlottown, PEI, Canada). A standard calibration curve was prepared by making a series of dilutions using diluent included with the DC-Cal cholesterol and TG reagent multi-calibrator (Diagnostic Chemicals Ltd.). Concentrations of calibration solutions were 4.84, 2.42, 1.21, 0.61, 0.30 and 0.15 mmol/L. The addition of 5 µl of each standard was transferred into disposable culture tubes (Fisher Scientific, Ottawa, ON, Canada) in triplicate. A 4x diluted control was prepared using 300 µl of diluent and 100 µl of DC-TROL Level 2 solution (Genzyme Diagnostic Ltd., Framingham, MA, USA) and 5 µl of the control was transferred into disposable culture tubes in triplicate. A total of 5 µl of lipid extract (25 mg/ml) was then transferred into disposable culture tubes in triplicate and dried down under a nitrogen evaporator. Five µl of diluent was added to dried-down tubes and a blank was prepared with 5 µl of diluent. Cholesterol reagent (500 µl) was added to all tubes and vortexed. Tubes were incubated for 20 minutes at room temperature and 200 µl was transferred into a Costar 96-well polystyrene assay plate (Corning Incorporated, Corning, NY, USA) and read at 505 nanometer using a FLUOstart Omega microplate reader (BMG Labtech, Ortenberg, Germany). The curve

and linear regression equation of standard solutions were used to calculate the concentrations of cholesterol within bison extracts.

4.4.1.4 Lipid separation: G-plate (Neutral lipids)

The method used for separation of neutral lipids (free fatty acids, triglycerides and phospholipids) is from Folch et al. (1957). Thin layer chromatography G-plate silica gel uniplates from Analtech Incorporated (Newark, DE, USA) were marked with six columns and activated at 110°C for at least 1 hour in an oven (Lab Line Instrument Inc., Melrose Place, IL, USA). A solvent system mobile phase was prepared with 80 ml petroleum ether, 20 ml diethyl ether and 1 ml acetic acid. The solvent was poured into a TLC tank (Fisher Scientific) containing filter paper and covered to allow the atmosphere to equilibrate. A standard solution was prepared in chloroform and methanol (1:1) using TG, cholesterol ester (CE), cholesterol, FFA and diglyceride (DG) in concentrations of 5 mg/mL for TG, cholesterol and DG and 1 mg/ml for CE and FFA. A total of 12 µl of standard was spotted using a Hamilton 50 µl syringe (Fisher Scientific) in the first column of the G-plate followed by 80 µl (2 mg) of bison lipid extract in the next five columns using a Hamilton 100 µl syringe (Fisher Scientific). A total of six G-plates were used to plate all bison lipid extracts. The syringe was cleaned between each extract using chloroform and methanol (1:1). Plates were placed in the chamber for 30 minutes or until solvent reached 1cm from the top of the plate. Plates were removed, air dried and sprayed with ANSA (0.1%) prepared in deionized water until covered. Plate were visualized under UV light using a UVGL-58 handheld UV lamp (UVP, Lipland, CA, USA). TG, FFA and PL bands were then marked with a pick, scraped onto weighing paper and

transferred into small methylated tubes (Fisher Scientific). Saponification of TG and methylation of TG, FFA and PL were performed as outlined in section 4.4.1.4 below.

4.4.1.5 Saponification and methylation

Saponification of TG samples was performed by adding 1.5 ml of 0.5N methanolic KOH to methylated tubes tightly sealed with teflon tape and heated in preheated dry sand using an Isotemp hot plate (Fisher Scientific, Dubuque, IA, USA) at 80-110°C for 1 hour and 30 minutes. Tubes were removed and allowed to cool for 10 to 15 minutes after which they underwent methylation.

Methylation of TG, FFA and PL samples was performed by adding 1.5 ml of BF_3 in methanol and 2 ml of hexane to tubes tightly sealed with Teflon tape and heated in preheated dry sand at 110°C for 1 hour. Tubes were removed and cooled before 1 ml of deionized water was added. Samples were capped and vortexed before being centrifuged for 10 minutes at 1300rpm. The upper phase containing hexane and methylated lipids was transferred to a 2 ml GC vial using a glass pasteur pipette.

TG and PL methylated samples were dried down under a nitrogen evaporator from approximately 1.8 ml to 1.0 ml and 0.5 ml, respectively. FFA methylated samples were dried down completely under the nitrogen evaporator, after which 175 µl of hexane was added before transferring into a glass insert. The insert was dried down and 175 µl was once again added to the original GC vial, transferred to an insert and dried down. A total of 20 ml of hexane was added to the insert containing FFA samples. All samples were vortexed and stored at -80°C until GC analysis.

4.4.1.6 Gas chromatography

Analysis was performed using a Varian 450-GC (Agilent Technologies, Walnut Creek, CA). An aliquot of the lipid solution (2.0 µl) was injected at 250°C in split mode (10:1) onto a Varian fused silica capillary 100m column (2.5mm ID). A filament delay of 5 minutes started each lipid run. The oven temperature was initially at 100°C for 2 minutes, increased at 25°C/minute to 175°C and held for 30 minutes, followed by an increase at 15°C/minute to 220°C and held for 10 minutes and increased at 20°C/minute to 240°C and held for 11 minutes. The total run time was 60 minutes using FID detectiom. The helium carrier gas flow rate was kept constant throughout the run at 1.8 ml/minute. Hexane was used as a blank and for autosampler wash.

4.4.1.7 Statistical analysis

One-way ANOVA was performed using a SPSS (Version 19) software program to analyze lipid data. Mean storage day differences for each compound were determined using Tukey's test with a significance level of $P<0.05$.

4.4.2 Results and Discussion

The total fat percentages in bison *LD* muscle averaged 3.34%, 2.45%, 2.61%, 2.97% and 2.60% at storage day 2, 4, 8, 15 and 21, respectively. Total lipid content and fat percentages for six bisons at 2, 4, 8, 15 and 21 days are shown in Table 4.4.1. No significant differences ($P<0.05$) in storage days were observed between the fat percentages of bison. The coefficient of variation (CV) rangeed from 39% to 51%, suggesting there is a large variation between animal fat percentages. The variation between replications suggests that the high CV is not caused by an error in extraction but

rather differences among animals. Bison 3 appears to have had the most total lipid and fat percentage particularly during storage days 2 to 8 compared to other animals. Although the six bisons consumed the same feed, were the same age, gender and species other factors such as stress, amount of feed consumed and genetic factors can influence the nutrient composition and affect the variation between animals. Several studies have reported the fat content of various muscle types in bison consuming either grain or grass-based diets. Reports on the *longissimus thoracis* (*LT*) muscle showed a fat content of 1.57% to 3.31% (Galbraith et al., 2006; Juárez et al., 2010; Koch et al., 1995; Marchello et al., 1998) which is similar to those reported for bison *LD* muscles in this study. Bison *LD* muscle also contains less fat than choice beef at 7.4% (Marchello et al., 1989). The total fat content of standard beef, pork and elk *longissimus* muscles as well as chicken breast has been reported as 2.7%, 4.9%, 4.0% and 0.7% respectively (Marchello et al., 1989; Rule et al., 2002). The total fat content of bison is therefore comparable to standard beef.

Table 4.4.1

Total lipids and fat percentages of bison LD muscles during post-mortem conditioning over 21 days at 4°C.

Conditioning Time (Days)[1]	Bison	Meat Extracted (g)	Total Lipids (g)	Fat %
2	1	1	0.0307	3.07
	1	1	0.0305	3.05
	2	1	0.0199	1.99
	2	1	0.0176	1.76
	3	1	0.0558	5.58
	3	1	0.0512	5.12
	4	1	0.0224	2.24
	4	1	0.0256	2.56
	5	1	0.0206	2.06
	5	1	0.0285	2.85
	6	1	0.0453	4.53
	6	1	0.0528	5.28
	Average			***3.34***
	SD			***1.40***
	CV (%)			***42%***
4	1	1	0.0254	2.54
	1	1	0.0230	2.30
	2	1	0.0158	1.58
	2	1	0.0132	1.32
	3	1	0.0540	5.40
	3	1	0.0451	4.51
	4	1	0.0248	2.48
	4	1	0.0216	2.16
	5	1	0.0203	2.03
	5	1	0.0209	2.09
	6	1	0.0158	1.58
	6	1	0.0144	1.44
	Average			***2.45***
	SD			***1.25***
	CV (%)			***51%***
8	1	1	0.0262	2.62
	1	1	0.0267	2.67
	2	1	0.0154	1.54
	2	1	0.0212	2.12
	3	1	0.0479	4.79
	3	1	0.0472	4.72
	4	1	0.0323	3.23
	4	1	0.0274	2.74

	5	1	0.0208	2.08
	5	1	0.0161	1.61
	6	1	0.0164	1.64
	6	1	0.0161	1.61
	Average			***2.61***
	SD			***1.14***
	CV (%)			***44%***
15	1	1	0.0312	3.12
	1	1	0.0222	2.22
	2	1	0.0190	1.90
	2	1	0.0196	1.96
	3	1	0.0318	3.18
	3	1	0.0361	3.61
	4	1	0.0562	5.62
	4	1	0.0432	4.32
	5	1	0.0299	2.99
	5	1	0.0311	3.11
	6	1	0.0179	1.79
	6	1	0.0179	1.79
	Average			***2.97***
	SD			***1.16***
	CV (%)			***39%***
21	1	1	0.0442	4.42
	1	1	0.0464	4.64
	2	1	0.0182	1.82
	2	1	0.0180	1.80
	3	1	0.0319	3.19
	3	1	0.0271	2.71
	4	1	0.0238	2.38
	4	1	0.0280	2.80
	5	1	0.0175	1.75
	5	1	0.0188	1.88
	6	1	0.0205	2.05
	6	1	0.0175	1.75
	Average			***2.60***
	SD			***1.02***
	CV (%)			***39%***

Note. [1]Different letters in the same row indicates a significant difference at $\rho<0.05$.

Results for cholesterol content in bison *LD* muscle is shown in Figure 4.4.1, with cholesterol averages of 82.69, 61.34, 68.07, 74.81 and 66.81 mg per 100 grams of meat at day 2, 4, 8, 15 and 21 days, respectively. No significant differences ($P<0.05$) in cholesterol was detected during conditioning. The method used for determination of cholesterol content in this study has not been used in previous studies of bison. Cholesterol content has been analyzed in bison by methods described by Stadman et al. (1957) and the Association of Official Analytical Chemists (AOAC) (1995). The current method was used to test the cholesterol-SL assay kit as an alternative method for analyzing cholesterol in meat tissue, however lipids did not dissolve properly suggesting this method is better used for plasma and other biological samples. In grass- and grain-fed bison, cholesterol content has been reported to range from 48.27 to 65 mg in the ribeye muscle (Galbraith et al., 2006; Marchello, 2001; Marchello et al., 1998).

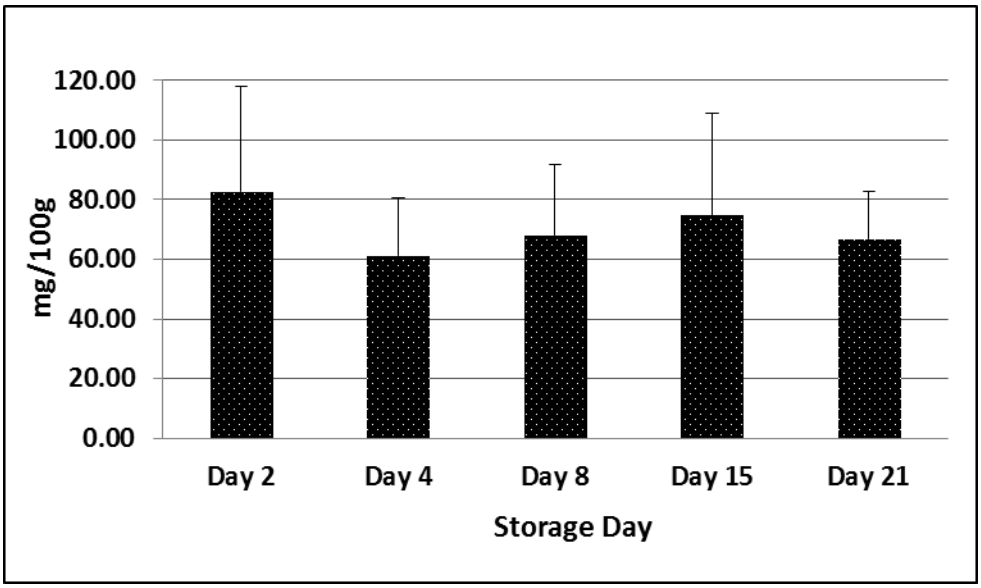

Figure 4.4.1 Cholesterol content (mg/100g of meat) in *LD* muscle of *Bison bison* during chilled storage conditioning for 21 days at 4°C. Values are means of 6 animals in triplicate with standard deviations.

An example of a FFA chromatogram of a bison extract is shown in Appendix L. Percentages of fatty acids for bison *LD* muscle are shown in Table 4.4.2. The most represented fatty acids in the lipids extracted from bison were stearic acid (C18:0), oleic acid (C18:1n9) and palmitic acid (C16:0) followed by linoleic acid (C18:2) and arachidonic acid (C20:4). Juarez et al. (2010) reported similar results in buffalo *LT* muscle with oleic, stearic, palmitic and linoleic being among the top contributors to the total fatty acid content of raw buffalo meat. Myristic and palmitic fatty acids are among the most atherogenic fatty acids (Spady et al., 1993), linoleic acid however, is thought to be neutral with respect to atherogenicity (Bonanome & Grundy, 1988). Atherogenesis is the formation of plaques made up of fat material (wiseGEEK, 2011). A significant difference ($P<0.05$) in storage days are seen in margaric acid (C17:0) between storage days 2 and 4 as well as a significant increase in eicosapentaenoic acid (EPA) (C20:5n3), linoleic and arachidonic acid between storage days 2 and 21. Arachidonic acid undergoes oxidation quickly making it an important source of volatiles during cooking (Mottram & Elmore, 1998) while high levels of linoleic acid have been shown to produce oily, sweet or bland tastes during cooking (Thu, 2006).

In the *LD* muscle of bison, fatty acid results showed that the saturated fatty acid content ranged from 56.34%, 61.74%, 54.95%, 55.05% and 49.74% at storage day 2, 4, 8, 15 and 21 days, respectively. The MUFA content ranged from 27.86%, 24.16%, 27.94%, 24.61% and 27.16% at storage day 2, 4, 8, 15 and 21 days, respectively. The PUFA content ranged from 15.32%, 13.85%, 16.33%, 19.76% and 22.33% at storage day 2, 4, 8, 15 and 21 in bison *LD* muscle, respectively. These results are similar for saturated fatty acids in the *LT* muscle of bison in which studies revealed a saturated fatty acid

content of 42.7% to 57.8%, however MUFA content was slightly lower and PUFA content was slightly higher than those reported (Juárez et al., 2010; Marchello, 2001; Marchello et al., 1998). The results for saturated, monounsaturated and polyunsaturated fatty acid are shown in Table.4.4.5, however some individual fatty acids were not detected in some animals.

Table 4.4.2

Percentages of free fatty acids in bison LD during post-mortem conditioning over 21 days at 4°C.

Free Fatty Acid	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
C14:0	0.44±0.36	0.30±0.25	0.36±0.26	0.34±0.27	0.42±0.16
C15:0	0.34±0.10	0.29±0.21	0.32±0.14	0.36±0.19	0.39±0.15
C15:1	0.38±0.47	0.20±0.23	0.43±0.81	0.29±0.23	0.45±0.45
C16:0	19.81±3.34	18.80±7.55	21.43±3.10	23.89±5.24	24.51±3.52
C16:1	1.29±0.81	0.82±0.60	0.93±0.42	1.16±0.73	1.28±0.57
C17:0	**1.90±0.70[a]**	**0.90±0.56[b]**	**1.16±0.16[b]**	**1.07±0.40[b]**	**0.99±0.13[b]**
C17:1	1.11±1.40	0.45±0.40	0.56±0.20	0.51±0.24	0.75±0.51
C18:0	26.19±8.68	22.46±7.83	24.82±4.97	21.91±2.65	19.95±4.30
C18:1(n-9)	23.79±4.35	20.05±6.86	22.74±3.27	23.21±4.93	22.83±3.52
C18:2	**6.61±4.25[a]**	**7.08±4.66[ab]**	**8.39±2.54[ab]**	**10.34±4.14[ab]**	**11.44±2.37[b]**
C20:4	**2.64±1.70[a]**	**3.68±2.30[ab]**	**4.27±1.52[ab]**	**5.10±2.13[bc]**	**5.84±1.24[c]**
C20:5n3	**0.18±0.19[a]**	**0.26±0.30[ab]**	**0.27±0.28[ab]**	**0.46±0.30[ab]**	**0.55±0.32[b]**

Note. Values are means of 6 animals in duplicate ± standard deviations.

[1] Different letters in the same row indicate a significant difference at $\rho<0.05$.

Similar fatty acids contributed to the total fatty acid content in the TG fraction. An example of a TG chromatogram of a bison extract is shown in Appendix M. Oleic acid had the highest total fatty acid percentage across storage days, followed by palmitic acid and stearic acid. Linoleic acid content was lower than that reported by the FFA fraction. No significant differences (P>0.05) were detected in triglycerides with the

exception of stearic acid that showed a significant difference (P<0.05) between storage day 2 and 8 as well as storage day 2 and 21. Table 4.4.3 shows the fatty acid breakdown of triglycerides over 21 conditioning days at 4°C. Other individual fatty acids were detected in TG bison samples, however individual fatty acids were not detected in some animals. No observable pattern suggesting that triglycerides are broken into FFA over conditioning at 4°C was evident as shown Figure 4.4.2.

Table 4.4.3

The composition of triglyceride fraction (% free fatty acids) in bison LD muscle during post-mortem conditioning over 21 days at 4°C.

Free Fatty Acid	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
C14:0	1.96±0.50	1.76±0.35	1.75±0.48	1.82±0.48	1.90±0.54
C15:0	0.45±0.13	0.36±0.14	0.36±0.15	0.40±0.08	0.42±0.12
C16:0	27.89±7.63	26.67±4.44	27.83±4.85	28.56±3.87	23.46±12.46
C16:1	1.88±1.37	2.13±1.22	1.96±1.24	1.80±0.1.18	1.97±1.46
C17:0	1.75±0.32	1.64±0.20	1.75±0.19	1.74 ±0.21	1.76 ±0.38
C17:1	1.04±0.35	0.95±0.21	0.77±0.41	0.96±0.19	0.96±0.32
C18:0	**11.21±9.84[a]**	**17.62±1.48[ab]**	**19.57±5.99[b]**	**15.12±7.28[ab]**	**19.21±2.88[b]**
C18:1(n-9)	47.69±9.07	43.30±3.23	37.31±14.07	44.19±6.96	45.09±8.86
C18:2	1.99±0.56	1.67±0.35	1.66±0.51	1.72±0.42	1.69±0.45
C18:3n3	0.39±0.09	0.32±0.13	0.29±0.16	0.34±0.09	0.33±0.07

Note. Values are means of 6 animals in duplicate ± standard deviations.

[1] Different letters in the same row indicate a significant difference at ρ<0.05.

In the TG fraction of the *LD* muscle of bison, fatty acid percentages for saturated fatty acid, MUFA and PUFA content is shown in Table 4.4.5. The results for fatty acids in the TG fraction is similar to those reported for saturated fatty acids in the FFA fraction as shown in Figure 4.4.3, however MUFAs are higher and PUFAs are lower as shown in

Figure 4.4.4 and Figure 4.4.5. Results for saturated fatty acids, MUFAs and PUFAs are similar to those repoted in the ribeye for bison in a study by Marchello et al. (1998).

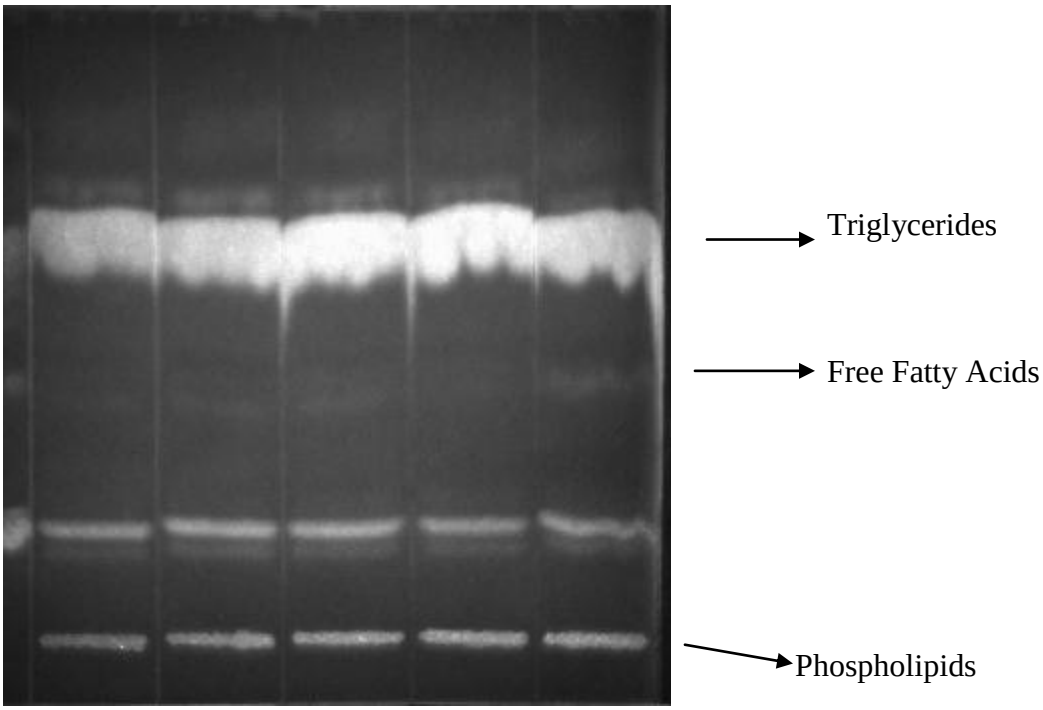

Figure 4.4.2 Example of bison G-plate separation under UV light. Five columns represent five different storage days from 2 days to 21 days (left to right).

Structural phospholipids have been shown to have an important role in flavour formation in cooked meat (Mottram & Elmore, 1998). An example of a PL chromatogram of a bison extract is shown in Appendix M. An overview of the fatty acids in the PL fraction are shown in Table 4.4.4. Phospholipids are important structural components in cells and contain a higher proportion of polyunsaturated fatty acids than triglycerides, particularly arachidonic acid, which makes them more susceptible to oxidation during heating (Mottram & Elmore, 1998). Arachidonic acid was higher than

the results shown in the FFA and TG fractions. While these compounds contribute to the flavour of meat, these lipid oxidation products have been shown to modify the profile of Maillard-derived volatiles produced in cooked meat (Mottram & Elmore, 1998). In the PL fraction, linoleic acid, palmitic acid, stearic acid, oleic acid and arachidonic acid contributed the greatest percentage to the total FFA content. High levels of linoleic acid as previously discussed, has been shown to produce oily, sweet or bland tastes during cooking (Thu, 2006). No significant differences were shown between storage days in the PL fraction of bison samples.

Table 4.4.4

The composition of phospholipid fraction (% free fatty acids) in bison LD muscle during post-mortem conditioning over 21 days at 4°C.

Free Fatty Acid	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
C14:0	1.36±1.24	1.18±1.13	1.32±0.94	1.31±1.55	1.42±1.31
C16:0	17.59±1.71	16.51±1.98	15.67±1.57	15.88±2.80	17.40±2.40
C16:1	1.51±0.42	1.38±0.42	1.32±0.26	1.38±0.36	1.45±0.23
C17:0	0.57±0.33	0.51±0.20	0.49±0.19	0.58±0.26	0.52±0.31
C17:1	0.43±0.40	0.52±0.31	0.50±0.32	0.48±0.34	0.44±0.37
C18:0	11.68±1.48	11.19±1.53	10.98±1.39	10.56±1.94	11.61±1.56
C18:1(n-9)	17.91±3.90	16.48±2.66	16.10±2.82	16.05±2.89	16.39±2.97
C18:2	22.75±3.93	24.58±2.51	24.69±1.89	22.51±2.67	24.55±2.83
C18:3n3	2.19±0.46	2.31±0.63	2.36±0.70	2.25±0.51	2.3±0.71
C20:4	10.29±1.91	10.13±1.58	10.26±1.05	9.40±1.58	9.28±3.59
C20:5n3	1.51±0.22	1.51±0.25	1.58±0.29	1.44±0.22	1.55±0.37
C22:5n3	2.52±0.43	2.44±0.28	2.60±0.33	2.32±0.35	2.52±0.46
C22:6n3	0.61±0.19	0.46±0.18	0.43±0.22	0.46±0.10	0.49±0.21

Note. Values are means of 6 animals in duplicate ± standard deviations.

[1] Different letters in the same row indicate a significant difference at $\rho<0.05$.

Table 4.4.5

The total saturated, monounsaturated and polyunsaturated fatty acids in bison LD muscle during post-mortem conditioning over 21 days at 4°C.

Lipid Fraction	Conditioning Time at 4°C (Days)[1]				
	2	4	8	15	21
Free Fatty Acid					
Saturated	56.34±7.08	61.74±12.59	54.95±6.14	55.05±8.16	49.74±5.03
Monounsaturated	27.86±6.40	24.16±9.63	27.94±3.62	24.61±10.61	27.16±7.25
Polyunsaturated	15.32±9.17	13.85±847	16.33±5.50	19.76±8.03	22.33±5.04
Triglycerides					
Saturated	43.71±10.71	48.48±5.46	51.65±9.79	48.20±7.21	47.16±10.41
Monounsaturated	52.95±9.85	48.51±5.09	41.87±15.07	48.97±6.58	50.00±9.64
Polyunsaturated	3.34±1.29	2.84±0.78	6.47±12.80	2.83±1.03	2.83±1.10
Phospholipids					
Saturated	37.15±3.04	36.22±2.28	36.23±3.20	39.25±2.13	36.97±3.81
Monounsaturated	21.70±4.27	20.88±2.75	20.41±3.24	20.94±2.78	20.77±3.39
Polyunsaturated	41.15±6.07	42.90±3.50	43.36±2.66	39.81±3.69	42.26±4.49

Note. Values are means of 6 animals in duplicate ± standard deviations, but some individual fatty acids were not detected in some animals.

[1] Different letters in the same row indicate a significant difference at $\rho<0.05$.

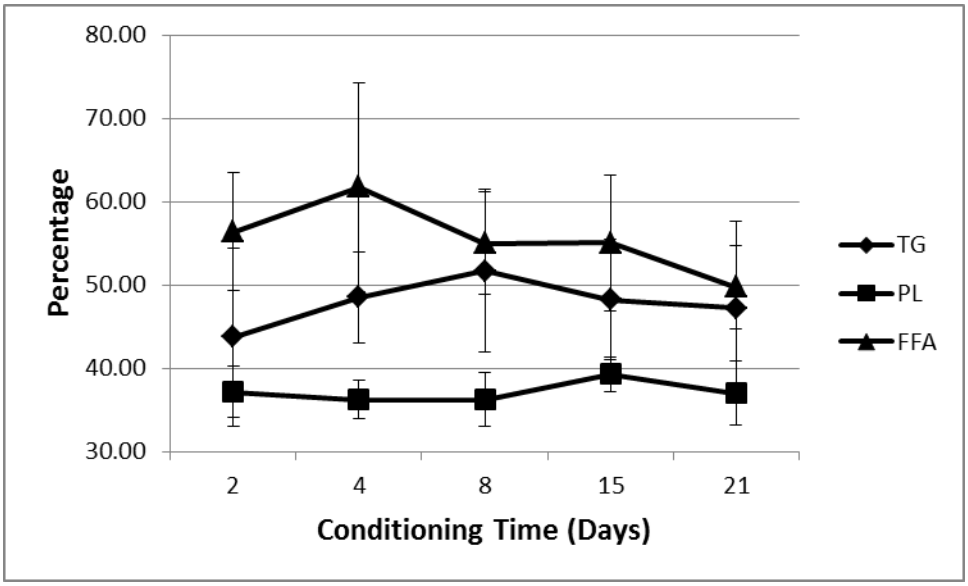

Figure 4.4.3 Saturated fatty acid percentages for FFA, TG and PL fractions in bison *LD* muscle at storage day 2, 4, 8, 15 and 21.

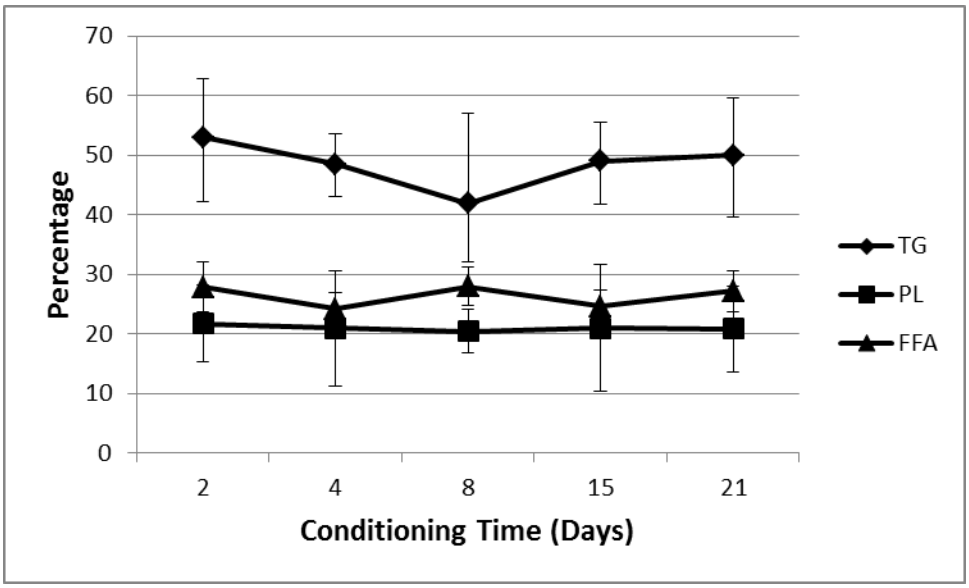

Figure 4.4.4 Monounsaturated fatty acid percentages for FFA, TG and PL fractions in bison *LD* muscle at storage day 2, 4, 8, 15 and 21.

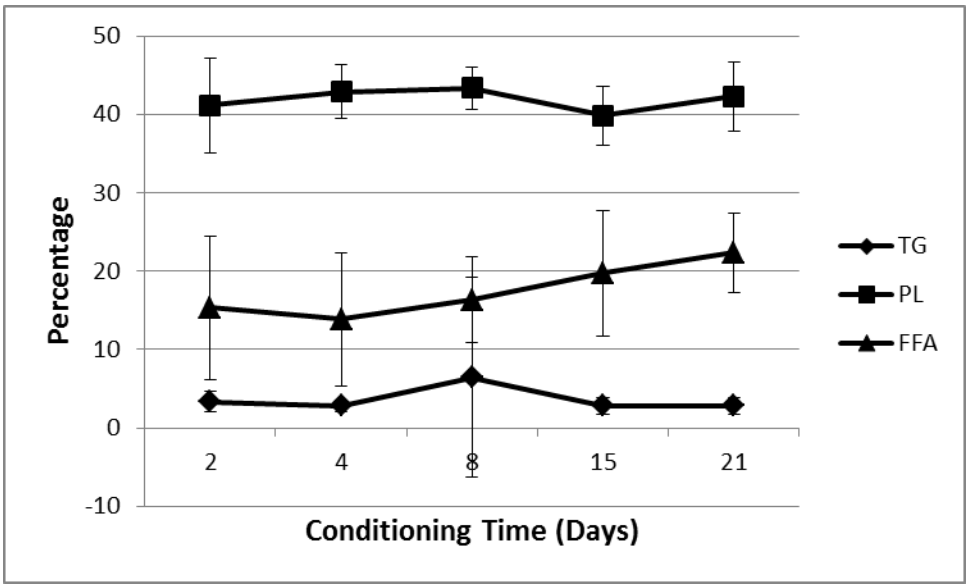

Figure 4.4.5 Polyunsaturated fatty acid percentages for FFA, TG and PL fractions in bison *LD* muscle at storage day 2, 4, 8, 15 and 21.

4.4.3 Conclusion

Lipid content is not only important due to its contribution to overall flavour, aroma and texture of bison but also for its associated health benefits. The Food and Drug Administration (1992) regulations label a serving of food (100g of bison meat) that contains less than 5 g of fat (5%) as low in fat (Food and Drug Administration, 1992). Stearic acid which is shown not to be a cholesterol-raising fatty acid (Kris-Etherton et al., 1993) was present in high amounts from FFA, TG and PL fractions in bison *LD* muscle during chilled storage conditioning over 21 days at 4°C. Myristic acid (C14:0) however is known to be a cholesterol raising fatty acid (Zock et al., 1994) and was present in low amounts.

Changes in fatty acid content in the FFA, TG and PL fractions during chilled storage conditioning at 4ºC over 21 days were almost non-existent, with the exception for a couple of fatty acids that were significantly different ($P<0.05$) in the FFA and TG fractions across chilled storage conditioning. Kesava Rao et al. (1991), found similar results in adult male buffalo, *triceps brachii* (*TB*), *LD* and *BF* muscles, in that storage period did not show any significant effect on total saturated, unsaturated and polyunsaturated fatty acids from phospholipids during refrigerated (4ºC) storage from 0 to 9 days. The predominant fatty acids in the phospholipids of the male buffaloes included palmitic, stearic, oleic and linoleic acid which is similar to the results found in bison *LD* muscle. Evidence of hydrolysis of phospholipids in bison *LD* muscle was not observed during storage, which may suggest little *phospholipase* activity during chilled storage conditioning. In the *LD* muscle of feedlot bison, Rule et al. (2002) reported

palmitic acid to be the highest which was also a major fatty acid contributor in the bison samples represented in this study.

Based on results reported in other studies, the muscle type and feeding regime of bisons are important to note. Range bisons have been emphasized to be the leanest bison meat and contain the lowest cholesterol concentration as well as most healthful fatty acid profiles (Rule et al., 2002) compared to feedlot bison. While grain-fed bisons are low in fat, grass-finished bison contains on average 5% more saturated fatty acids, 6% more PUFAs and 11% less MUFAs than grain-finished bisons (Marchello, 2001).

A discussion of the overall water soluble and lipid-soluble results as well as the correlation of FFA, TG and PL percentages to sensory evaluation results will be discussed in Chapter 5 – Correlation Studies.

Chapter 5 – Correlation Studies

Correlations between reducing sugars and sugar phosphates, free amino acids, nucleotides, nucleosides and bases as well as lipid concentrations with sensory evaluation results were performed using XLSTAT 2011 statistical analysis software (Addinsoft, New York, NY, USA). Partial least squares (PLS) regression was used to correlate chemical analysis results with sensory evaluation results ($P<0.05$). PLS is a modeling method that tries to find the fundamental relationship between two matrices. In the case of this study it takes the dependent variable which is the standardized mean sensory evaluation result from three replications and eight panelists (Y) and tries to correlate it with the explanatory variable (in this case the selected flavour precursors concentration descriptors at the same storage day). PLS is an extension to the multiple linear regression model; however it does not impose the restrictions employed by discriminant analysis, principal component regression, and canonical correlation (StatSoft, 2002). PLS takes into account both *X* and *Y* variables, whereas when PCA was used for sensory evaluation analysis in section 3.6 only the *X* variable was taken into account. To help visualize the results and get an overall understanding of the variation between water-soluble flavour precursors as well as lipid results with sensory attributes across storage days 2, 4, 8, 15 and 21, PLS was used.

5.1 Correlation between Sugar and Sugar Phosphate Composition and Sensory Analysis

The model of quality of number of components for the correlation between sugar and sugar phosphate composition and sensory analysis (shown in Figure 5.1.1) showed a low value for Q^2 cumulated index which is the measure of global goodness of fit and the

predictive quality of the sensory evaluation results, indicating that the quality of fit varied considerably depending on the sensory attribute. The cumulative R^2Y and R^2X correspond to the correlations between the explanatory (*X*) and dependent (*Y*) variables and are very close to 1, indicating that the 3 components generated by the PLS regressions summarize well both the *X* and *Y* variables.

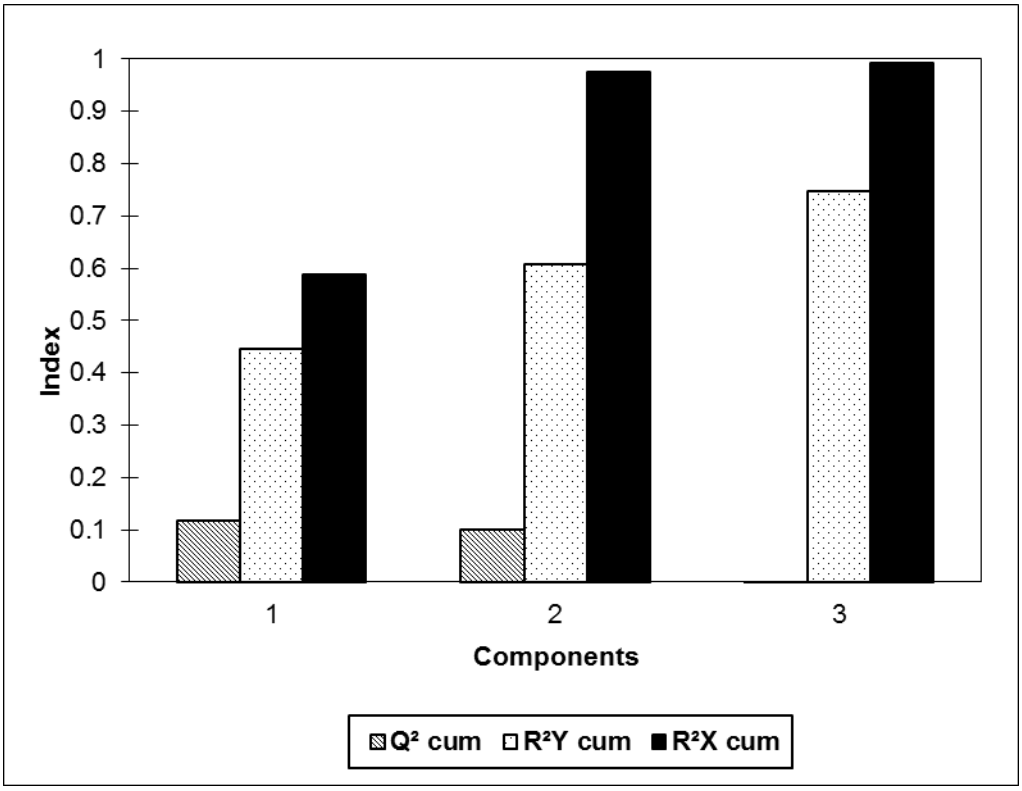

Figure 5.1.1 Model quality for number of components for correlating sugar and sugar phosphate concentrations and sensory evaluation results. An index close to 1 is ideal.

The correlation between reducing and phosphate sugars and sensory evaluation analysis was 59.95%. Figure 5.1.2 shows the correlation of *X* (sugars) and *Y* (sensory evaluation) variables. The vector t2 represents the most variation in Y, while vector t1 represents the variation in X, however it does not necessarily explain the most variation

in X. Figure 5.1.2 shows the score plot between t1 and t2. As expected, storage day 2 and 4 are clearly separated from storage day 15 and 21. Storage day 8 also appears to be separated from both storage day 2 and 4 as well as 15 and 21 days. The sensory evaluation attributes of chewiness and connective tissue were time dependent as seen in Chapter 3 and appear to be correlated with storage day 2 and 4 along with oily/fatty aroma. Beef, sour, salty and oily/fatty flavour, vinegar/sour aroma as well as oily mouth coating are correlated with storage day 15 and 21. Initial and overall tenderness as well as juiciness are closely correlated with storage day 8 and 15. These correlations of sensory attributes to storage day are similar to those seen with PCA results shown in Figure 3.1.6.

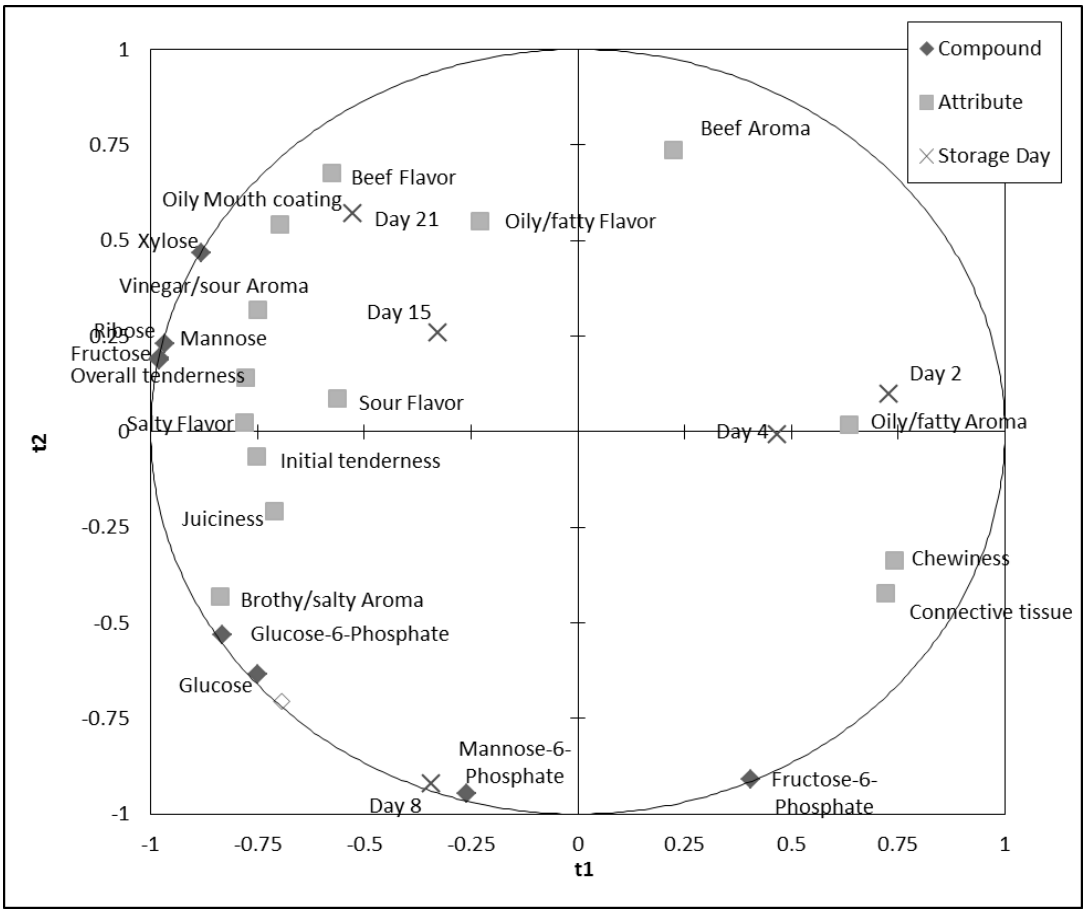

Figure 5.1.2 Correlations of sugars and sugar phosphates with sensory attributes and storage days on axes t1 and t2.

5.2 Correlation between Amino Acid Composition and Sensory Analysis

The model of quality of number of components for the correlation between amino acid composition and sensory analysis is similar to that of sugar and sugar phosphates. The correlation between amino acids and sensory evaluation results is 67.73%. Figure 5.1.3 shows the correlation between *X* and *Y* variables and the five conditioning days. The separation of between storage days and time dependent nature of the flavour, aroma and texture attributes from sensory evaluations are similar to those seen in Section 5.1 above. Once again the majority of amino acids are correlated with storage days 15 and 21. Alpha-aminopimelic acid, ornithine and especially glutamine are not correlated to any one storage day in particular which is also supported by the mean results shown in Table 4.3.1 in which these three amino acids are not significantly different during storage days at a significance level of 0.05. The PLS results show a strong correlation between proline and storage day 21. The correlation with the majority of amino acids and storage day 15 and 21 may suggest that these amino acids contribute to the beef, oily/fatty, salt and sour flavour, vinegar/sour and brothy/salty aroma as well as oily mouth coating, initial tenderness, overall tenderness and juiciness of bison meat.

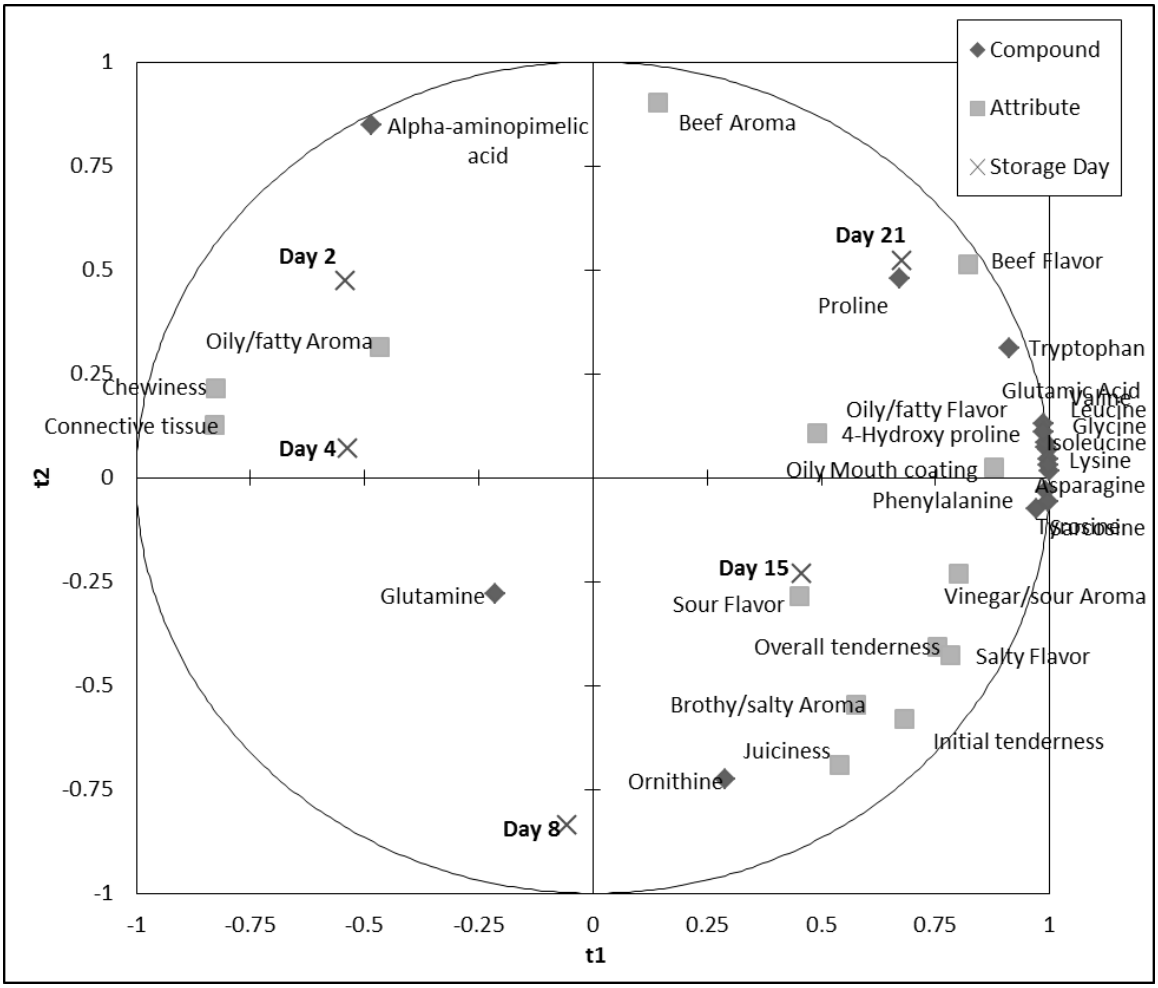

Figure 5.1.3 Correlations of amino acids with sensory attributes and storage days on axes t1 and t2.

5.3 Correlation between Nucleotide, Nucleoside and Base Composition and Sensory Analysis

The model of quality of number of components for the correlation between nucleotide, nucleoside and base composition and sensory analysis is similar to that of sugars and amino acids. However, the cumulative R^2Y and R^2X that correspond to the correlations between the explanatory (*X*) and dependent (*Y*) variables were closer to 1. Therefore the 3 components generated by the PLS regressions summarize the *X* and *Y*

variables better than with reducing sugars and amino acids. The correlation between nucleotides, nucleosides and bases with sensory evaluation results is 68.35%.

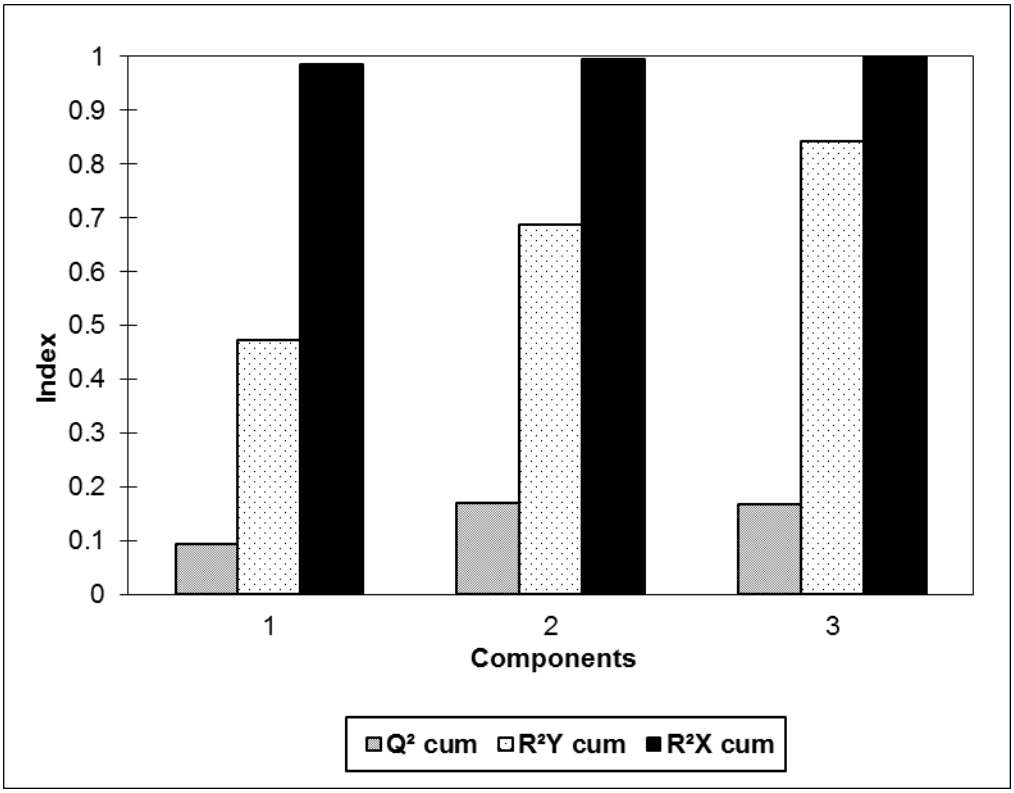

Figure 5.1.4 Model quality for number of components for correlating nucleotide concentrations and sensory evaluation results. An index close to 1 is ideal.

Figure 5.1.5 shows the correlation between *X* and *Y* variables and the five conditioning days. The storage day separation in Figure 5.1.5 show storage day 2 and 4 to be less significant, while 4 and 8 were more significant compared to the separation seen in the PLS results for sugars and amino acids. The sensory attributes were correlated similarly with those seen in section 5.1 and 5.2 however, oily/fatty aroma was positively correlated with storage day 2. As expected, IMP was strongly correlated with storage day 2 and 4, while its breakdown products, hypoxanthine and inosine were shown to be strongly correlated with storage day 15 and 21, thus suggesting that IMP was being

broken down to hypoxanthine and inosine during chilled storage at 4°C, over 21 days of conditioning as seen in Table 4.3.1. Figure 5.1.3 also showed ribose to be correlated with storage day 15 and 21, thus also suggesting IMP may be broken down further to ribose during conditioning.

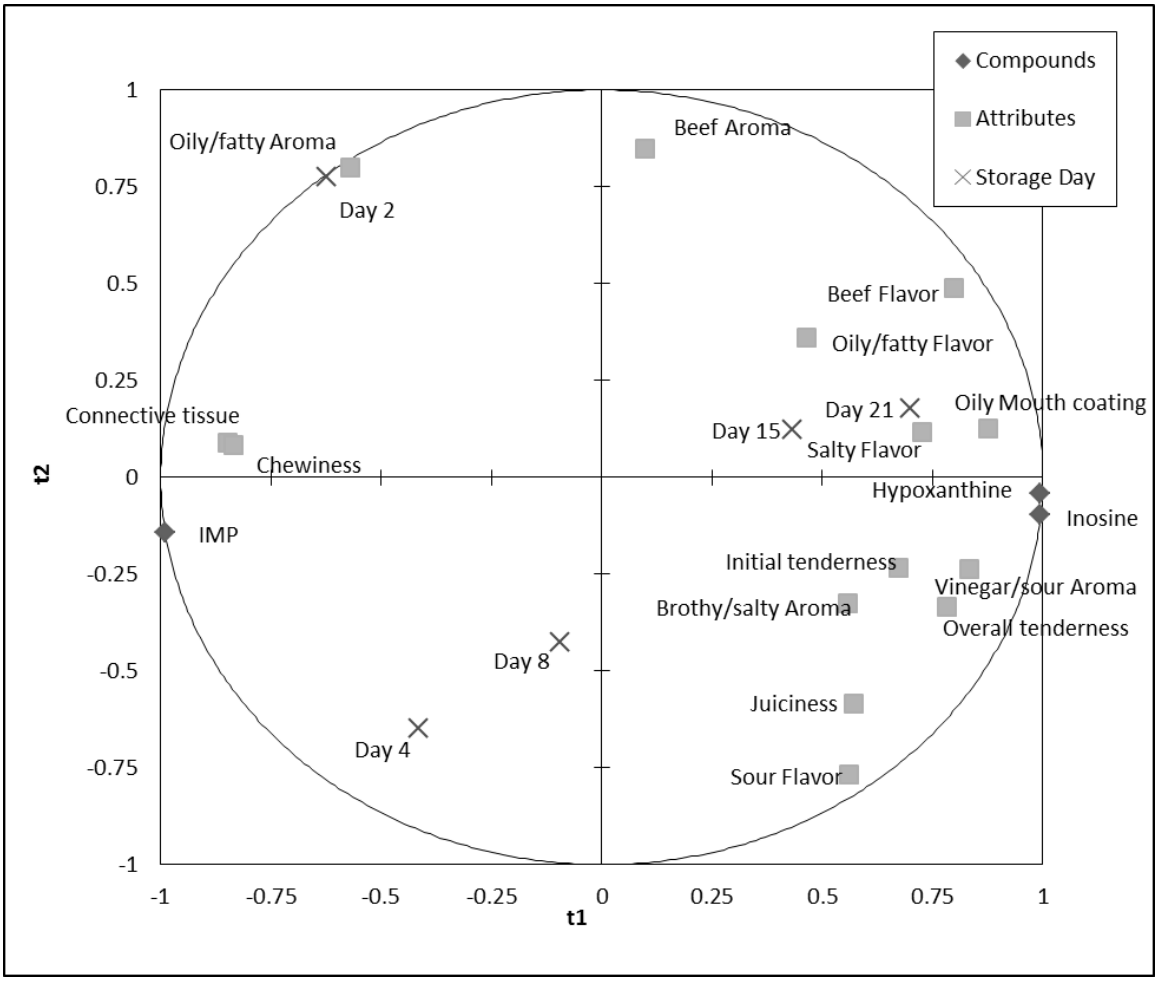

Figure 5.1.5 Correlations of nucleotides, nucleosides and bases with sensory attributes and storage days on axes t1 and t2.

5.4 Correlation between Lipid Composition and Sensory Analysis

The correlation between lipids and sensory evaluation results was 78.34%, which is higher than the correlation between water-soluble components and sensory evaluation results. Figure 5.1.6 shows the correlation between *X* and *Y* variables and the five conditioning days. The separation of between storage days and time dependent nature of the flavour, aroma and texture attributes from sensory evaluations were separated similarly to the separation of nucleotides, nucleosides and base, with storage day 4 and 8 more closely related. The sensory attributes of chewiness and connective tissue were positively correlated with day 4, while oily/fatty aroma was positively associated with storage day 2. Storage day 15 and 21 were strongly associated (as seen in PLS results for sugars, amino acids and nucleotides), with sensory attributes, oily/fatty, salty and beef flavor, oily mouth coating, initial tenderness, overall tenderness and vinegar/sour aroma were positively correlated with these conditioning times. Juiciness, sour flavour and brothy/salty aroma were negatively correlated with any one storage day in particular.

In regards to the lipid correlation to storage day and sensory attributes PLS results show PUFA, palmitic acid, linoleic acid and arachidonic acid from the FFA fraction were correlated with storage day 15 and 21, along with total saturated fatty acid content from the phospholipid fraction. Stearic acid, saturated fatty acid and PUFA from the TG fraction and linoleic acid and PUFA from the PL fraction of bison samples were positively associated with storage day 8. Total MUFA, saturated and stearic fatty acid contents from the FFA fraction and palmitic acid from the TG fraction were correlated with storage day 4. All other fatty acids from all three fractions were correlated with storage day 2. An observable correlation pattern is shown between oleic acid from all

three fractions with storage day 2. It also appears that more fatty acids are associated with day 2 compared with other storage days. Phospholipids appear to be correlated with storage day 2, while FFA are strongly correlated with storage day 15 and 21.

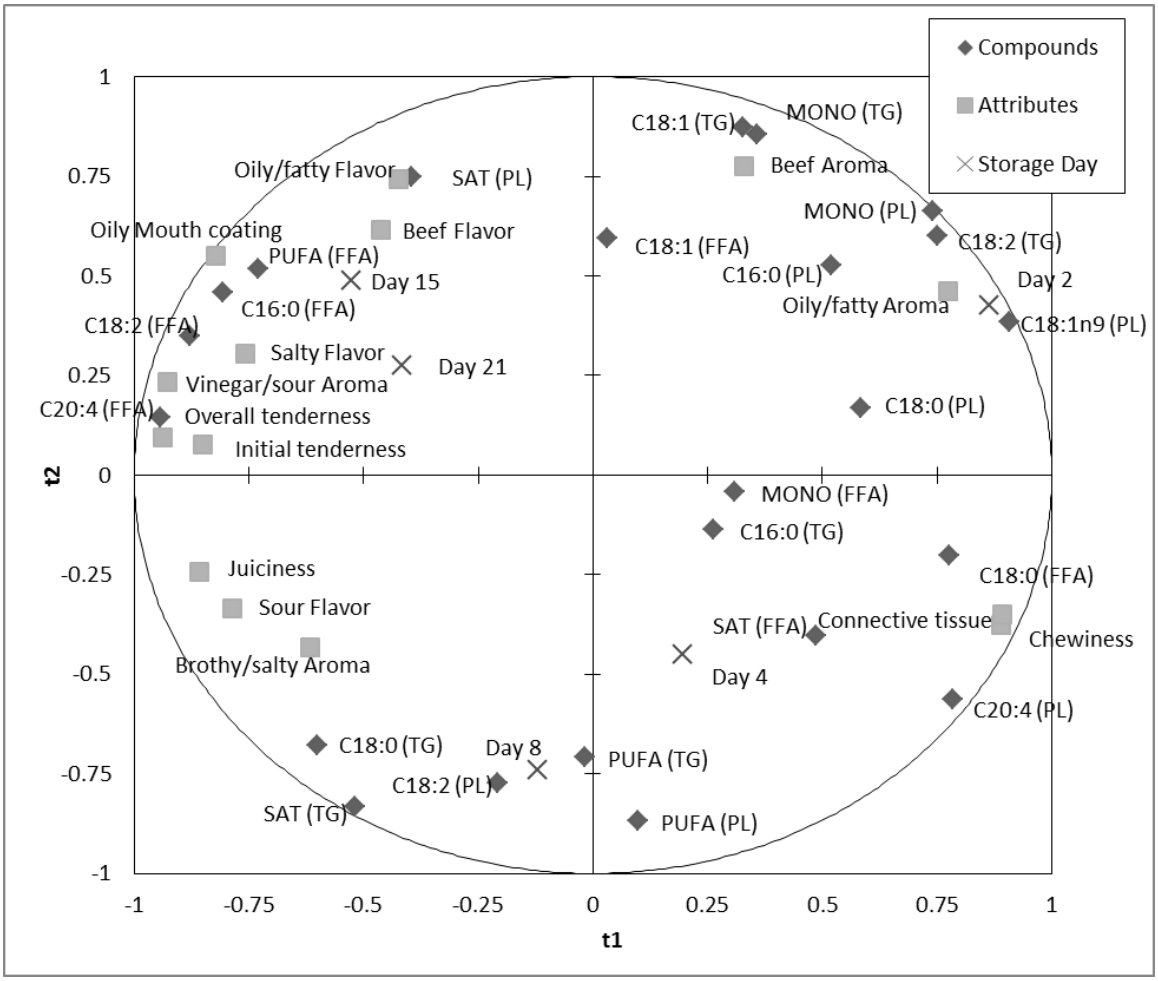

Figure 5.1.7 Correlations of fatty acids from FFA, TG and PL fractions in bison with sensory attributes and storage days on axes t1 and t2.

As expected, correlations of water-soluble compounds including sugars and sugar phosphates, free amino acids, nucleotides, nucleosides and bases with sensory evaluation

results showed that specific flavour precursors are time dependent and correlate positively with associated sensory attributes. The correlation percentage between lipid results and sensory evaluation results was higher than those seen with water-soluble flavour precursors however, the separation between specific fatty acids from FFA, TG and PL fraction in bison samples showed no significant differences. Specific fatty acids did not appear to be time dependent which was also seen in SPSS statistic results in which no significant differences were detected between most fatty acids across storage days.

Chapter 6 – General Discussion

The effects of chilled storage conditioning at 4°C on the concentrations of key water- and lipid-soluble meat flavour precursors were monitored in *Bison bison LD* muscle at 2, 4, 8, 15 and 21 days *post-mortem*. Results revealed some significant changes that occurred in flavour precursors namely sugars, sugar phosphates, free amino acids, nucleotides, nucleosides and bases with extended conditioning for 21 days in bison *LD* muscle, which could potentially affect the formation of flavour compounds upon heating. The *LD* muscle of bison during chilled storage conditioning has also influenced the sensory qualities of the cooked meat using quantitative descriptive analysis. The sensory attribute changes in *post-mortem* bison meat have been correlated with the chemical analysis of flavour precursors stored at 4°C to compare any flavour trends optimized during cooking. Flavour precursor concentrations were positively and negatively correlated with sensory attributes and storage days and revealed some interesting relationships.

Several significant differences ($P<0.05$) have been detected across storage days for many water-soluble flavour precursors. A significant increase ($P<0.05$) in storage day for sugars including ribose, xylose and mannose, in most amino acids including sarcosine, glycine, valine, leucine, isoleucine, asparagine, 4-hydroxy proline, glutamic acid, phenylalanine, lysine, tyrosine and tryptophan, in the nucleoside inosine and base hypoxanthine as well as a significant decrease in IMP concentrations were observed in bison *LD* muscle extracts. Positive correlations with most sugar and sugar phosphates as well as free amino acid concentrations with storage day 15 and 21 have been found by PLS analysis of results from bison *LD* muscle.

Quantitatively, the most significant changes ($P<0.05$) observed amongst the water-soluble flavour precursors during conditioning have been the increases in fructose, valine, isoleucine, mannose, tyrosine and leucine. Glucose and glutamine concentrations were high in bison *LD* muscle which was similar to results reported in beef (Koutsidis et al., 2008a) and pork (Meinert et al., 2009) that showed glucose and glutamine to be the highest contributors of reducing sugars and free amino acids. Although amino acids contribute various individual flavour components, in combination they may produce a desirable amino acid flavour profile. Cysteine and methionine are known to be important flavour precursors, particularly in their contribution in the Maillard reaction, and were not detected in bison extracts, however previous studies on flavour precursors in beef (Koutsidis et al., 2008a) only detected these compounds in trace amounts.

Nucleotides, nucleosides and bases showed some interesting results in bison *LD* muscle with a significant decrease ($P<0.05$) in contents of IMP and the accumulation of its breakdown products, hypoxanthine, inosine and ribose. IMP was negatively correlated with storage days 15 and 21, while its degradation products were positively correlated with storage days 15 and 21. This suggests that the enzyme *inosine nucleosidase* which catalyzes the breakdown of inosine to ribose and hypoxanthine is an important pathway in the degradation AMP during chilled storage conditioning of bison. Evidence of IMP being hydrolyzed by *inosinate nucleosidase* to form R5P is non-existent however, R5P and R1P (which are formed by *purine-nucleosidase phosphorylase*) may have been broken down before conditioning day 2. It is expected that the breakdown of inosine to hypoxanthine and ribose should produce equal moles of each product, however

concentrations of hypoxanthine were much higher than ribose, which suggests an alternate route for the production of hypoxanthine is responsible.

Very few significant differences ($P<0.05$) were detected across storage days in fatty acid percentages in FFA, TG and PL fractions of bison *LD* muscle. High concentrations of polyunsaturated fatty acids in the PL fraction of bison lipid extracts will contribute to the greater quantity of volatile products formed due to their increased susceptibility to oxidative breakdown during cooking than monounsaturated and saturated fatty acids. Saturated and monounsaturated fatty acids were detected in higher concentrations in the FFA and TG fractions, respectively. Stearic, oleic, palmitic, linoleic and arachidonic acids were all major contributors to the three fractions of lipids with the exception of linoleic and arachidonic acid in TG fractions of bison *LD* muscle. Although no significant differences were detected in saturated, monounsaturated and polyunsaturated fatty acids, lipids can generate flavour active compounds that contribute to meat flavour upon thermal oxidation and can also interact with other components in tissues to create other flavour compounds. Lipids can also act as a solvent for several aroma compounds such as cysteine to minimize their aroma potential and help generate more desirable meat flavours (Mottram, 1996; Wasserman, 1972). Bison *LD* muscle was shown to be low in fat with percentages averaging less than 3.4%. When choosing alternative meat products such as bison, health benefits are likely one of the components at the forefront of consumer behavior.

Flavour is the most important sensory attribute to the overall acceptance of meat (Bryhni et al., 2002). As expected, the *LD* muscle of bison during chilled storage conditioning (4°C) has influenced the sensory qualities of the cooked meat using

quantitative descriptive analysis. Significant differences ($P<0.05$) were observed between day 2 and 21 for vinegar/sour aroma, initial tenderness, juiciness, overall tenderness, connective tissue and chewiness. A relationship between beef aroma and flavour with day 21 as well as chewiness and connective tissue with day 2 and 4 were observed in the STATIS PCA bi-plot.

6.1 Significance of Research

The significance of this research is that this is the first time that the effect of chilled storage conditioning on key flavour precursors such as reducing sugars and sugar phosphates, free amino acids, nucleotides, nucleosides and bases were investigated in bison meat. Since bison is a fairly common meat consumed in Canada, this research can benefit producers and consumers throughout Canada regarding flavour developments in this type of meat. Identification of water-soluble and lipid-soluble flavour precursors is important due to their potential impact on flavour formation in standard food production. This research will have a potential impact on the meat industry by providing valuable knowledge through the identification of limiting precursors in local meats such as bison which will improve the marketability of these products nationally, while benefiting producers and consumers. Examining the effect of *post-mortem* conditioning through chilled storage will enable the meat industry to understand the changes occurring in biochemical pathways in bison meat and the potential impact of these changes on the flavour of meat. Flavour chemistry can help the meat industry find ways to optimize the eating quality of meat in their quest to provide healthy and functional foods.

6.2 Recommendations for Future Research

Upon completion of this research, several follow-up studies should be conducted to further enhance and expand on the information provided for bison *LD* muscle during chilled storage conditioning over 21 days. My first suggestion would be to increase the number of animals (n>10) in order to reduce animal to animal variation. Six animals were used in other studies and the expectation was that the variation would be the same however based on my results bisons show more variation between animals. Second, I would suggest using an alternative cooking methods in order to generate a roasty meat flavour contributed by the Maillard reaction. The choice of cooking method used for this study, although acceptable to detect differences between storage days did not generate roasty aromas and flavours considered desirable in meat products. Grilling bison steaks is a recommendation for future research on the sensory quality of bison meat.

Another limitation of the current study is that regulations prevented the analysis of flavour precursors before 48 hours. My recommendation for future research, if possible, would be to test flavour precursors at an earlier conditioning time than 2 days post-slaughter, in order to evaluate the potential compounds that degrade quickly such as ATP, cysteine, creatine, creatinine and possibly R1P. Earlier conditioning analysis will hopefully help identify some of the biochemical reactions responsible for the formation of certain flavour precursors such as the pentose phosphate pathway, glycolysis and the AMP breakdown pathway. The AMP breakdown pathway however could not explain the formation of sugars such as ribulose-5-phosphate, R5P, xylulose or the formation of other compounds such as myo-inositol and myo-inositol-phosphate in chicken (Aliani, 2008). The origin of flavour precursors is important for the overall understanding of meat

flavour. Alternative methods for detecting cysteine, methionine, creatine and creatinine should also be considered as trace amounts were undetectable in bison *LD* muscle. These compounds have been reported to contribute to the flavour and aromas of cooked bison meat. The investigation of chemical reactions and their volatile products based on the changes in flavour precursor concentrations shown in bison *LD* muscle would also be beneficial for future research in order to further identify the specific flavour precursors responsible for the desirable or undesirable sensory attributes.

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APPROVAL CERTIFICATE

June 16, 2010

(NSERC)

TO: **Michel Aliani**
Principal Investigator

FROM: **Wayne Taylor, Chair**
Joint-Faculty Research Ethics Board (JFREB)

Re: **Protocol #J2010:093**
"Sensory Evaluation of Bison Steak and Chicken Breast and Thigh"

Please be advised that your above-referenced protocol has received human ethics approval by the **Joint-Faculty Research Ethics Board**, which is organized and operates according to the Tri-Council Policy Statement. This approval is valid for one year only.

Any significant changes of the protocol and/or informed consent form should be reported to the Human Ethics Secretariat in advance of implementation of such changes.

Please note:

- **if you have funds pending human ethics approval, the auditor requires that you submit a copy of this Approval Certificate to Eveline Saurette in the Office of Research Services, (e-mail eveline_saurette@umanitoba.ca, or fax 261-0325), including the Sponsor name, before your account can be opened.**

- **if you have received multi-year funding for this research, responsibility lies with you to apply for and obtain Renewal Approval at the expiry of the initial one-year approval; otherwise the account will be locked.**

The Research Ethics Board requests a final report for your study (available at: http://umanitoba.ca/research/ors/ethics/ors_ethics_human_REB_forms_guidelines.html**) in order to be in compliance with Tri-Council Guidelines.**

Bringing Research to Life

Panelist # _____

Evaluate the samples in the following order.

_____ _____ _____ _____ _____

Rinse your mouth with water completely before evaluating each sample.
Take the first sample listed above and evaluate the intensity of the aroma attributes first followed by the texture and flavor attributes according to the methods described. After all of the attribute intensities have been marked continue in the same manner for the 2nd and subsequent samples. To record your response, place a vertical line across the horizontal line which corresponds to the intensity of the attribute and write the code number above it.

Aroma

Place the sample container in position for sniffing.
Remove the cover.
Take three short sniffs and replace the cover.

Flavor
Place one piece of sample in your mouth.
Chew the sample thoroughly.
Evaluate the flavor attribute intensities just before swallowing the sample.

Beef Aroma – Aroma associated with stewed beef.

_____
low high

Vinegar/sour Aroma – Aroma associated with vinegar.

_____
low high

Brothy/salty Aroma – Aroma associated with beef broth powder.

_____
low high

Oily/Fatty Aroma – Aroma associated with cooked regular ground beef.

_____
low high

Appendix B

Panelist # _____

Evaluate the samples in the following order.

_____ _____ _____ _____ _____

Initial tenderness - Take the piece of meat with the fork and place it so that the two ends of the sample are between your molars. Take one bite. Evaluate the ease of biting through the sample.

_____

low high

Juiciness – Chew the sample 2 times. Evaluate the amount of moisture present in the sample.

_____

low high

Overall tenderness – Chew the sample 4 to 5 times. Evaluate the **ease of chewing** the sample.

_____

low high

Connective tissue - Chew the sample 4 to 5 times. Evaluate the amount of connective tissue present in the sample.

_____

low high

Beef Flavor – Flavor associated with broiled rib eye steak.

_____

low high

Sour Flavor – Flavor associated with citric acid in water.

_____

low high

Salty Flavor – Flavor associated with sodium chloride in water.

_____

low high

Appendix B

Panelist # _____

Evaluate the samples in the following order.

_____ _____ _____ _____ _____

Oily/Fatty Flavor – Flavor associated with cooked regular ground beef.

_____

low high

Chewiness - Chew the sample completely. Evaluate the length of time required to prepare the sample for swallowing.

_____

low high

Oily mouth coating – Chew the sample completely. Evaluate the amount of oily film remaining in the mouth after swallowing.

_____

low high

Comments:

Appendix C

Recruitment Email

Hello Everyone,

Here is another opportunity to become involved in Food Sensory Research. Aroma, flavor and texture attribute analysis of bison steak is the focus. This involves both the training and the actual experimental sessions which will be nine in total. The only criteria are that participants have no food allergies. An honorarium is provided. Details are outlined in the attached information package which includes a letter, consent form and questionnaire. The panel sessions will be held in the Weston Sensory Centre Fourth Floor Human Ecology Building. A response by e-mail or telephone (see below) by Tuesday, October 19 would be greatly appreciated as we are planning the first session for Wednesday, October 20 from 11:30 to 12:15. I am happy to answer any questions and provide more information. Forwarding this e-mail to others that may be interested would be greatly appreciated.

Department Office Assistants and Sheri: Could you please forward this e-mail to sessional instructors and graduate students respectively?

Thank you very much for your time and consideration.

Sincerely,
Donna Ryland, Department of Human Nutritional Sciences
Weston Sensory and Food Research Centre
204-474-8071 (telephone) 204-474-7592 (fax)
ryland@cc.umanitoba.ca (e-mail)
and
Dr. Michel Aliani, Department of Human Nutritional Sciences
Weston Sensory and Food Research Centre
204-474-8070 (telephone) 204-474-7592 (fax)
aliani@cc.umanitoba.ca (e-mail)

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October 5, 2010

Dear Colleague,

We are recruiting volunteers to participate in a research study on the aroma, flavor and texture of bison steak which is sponsored by the Natural Sciences and Engineering Research Council of Canada. You would have the opportunity to learn how panelists are trained to measure the intensity of specific aroma, flavor and textural attributes of this product. A potential risk would be allergic reactions to food products. Due to this risk people with food allergies will not be allowed to participate. This letter explains what your commitment would be. If you have any questions please call me, Donna Ryland at 474-8071 or e-mail ryland@cc.umanitoba.ca.

Eight to ten panelists will take part in group sessions (training component) followed by sessions where evaluation is done individually. Training involves group discussion of definitions and techniques for measuring flavor and textural attributes and evaluation of their intensities in various samples. Other food products may be used to facilitate these discussions and aid in the development of the measuring instrument. There will be six training sessions and three individual sessions for a total of 9 sessions held on average three times a week at 11:30 to 12:15. The first meeting is tentatively planned for Wednesday, October 20 when times and dates for future sessions will be confirmed with the group. A gift card from the University of Manitoba Bookstore for $50.00 will be given to those completing all of the required sessions. The study will take place on the Fourth Floor in the Human Ecology Building.

Completion of the enclosed questionnaire will confirm that no food allergies exist. If you are interested in helping us with this research notify Donna at 474-8071 or e-mail ryland@cc.umanitoba.ca on or before Tuesday, October 19. Please complete the attached consent form and questionnaire confirming that no food allergies are present and e-mail them back before the first meeting. This research has been approved by the Joint-Faculty Research Board of Ethical Review at the University of Manitoba. If you have any concerns or complaints about this project, you may contact the Human Ethics Secretariat at 474-7122.

We hope that you will be able to take part in this research and look forward to hearing from you. Alternatively, if you know of anyone else that might be interested in participating we would appreciate it if you could forward this information to them. Thank you very much.

Sincerely,

Donna Ryland, Research Assistant, and
Dr. Michel Aliani, Assistant Professor
Department of Human Nutritional Sciences

UNIVERSITY OF MANITOBA

Faculty of Human Ecology
Department of Human Nutritional Sciences

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CONSENT FORM

Research Project Title: **Sensory Evaluation of Bison Steak**
Sponsored by: **Natural Sciences and Engineering Research Council of Canada**
Researchers: Dr. Michel Aliani, Department of Human Nutritional Sciences
Donna Ryland, Department of Human Nutritional Sciences

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The study is being done to evaluate the aroma, flavor and textural attributes of bison steak. Six training sessions will be conducted where panelists meet as a group to learn the aroma, flavor and texture attribute definitions as well as the scale used to measure the intensity of the attributes. Samples of bison steak as well as other products useful in defining specific attributes will be tasted to familiarize panelists with procedures. Three experimental sessions will be held in individual panelist booths. All sessions will be approximately 45 minutes and take place on the Fourth Floor of the Human Ecology Building about three times per week. Therefore the total maximum time commitment will be about 7 hours (9 sessions at 45 minutes each) over a three to four week period.

Possible risk may be allergic reactions to food products eaten. Due to this risk people with food allergies will not be allowed to participate. Completion of the accompanying questionnaire will confirm that no food allergies exist.

Panelists will be identified by number and all data related to personal information and results obtained will be kept in a locked cabinet in Room 400 Human Ecology Building for 5 years after the date of ethics approval or until data are published whichever comes first. Access to information linking panelist to number will be limited strictly to the researchers named above. All data will be shredded after the time has expired. Data published will be given as group means with no individual names given.

A gift card from the University of Manitoba Bookstore for $50.00 will be provided for those completing all of the required sessions.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release researchers, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time, and/or refrain from answering any questions you prefer to omit, without prejudice or consequence. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. This study is being conducted by Dr. Michel Aliani, Assistant Professor, Department of Human Nutritional Sciences, telephone – 474-8070, e-mail – aliani@cc.umanitoba.ca. A telephone number and e-mail address is requested in case unforeseen circumstances necessitate a change in the schedule.

This research has been approved by the Joint-Faculty Research Board of Ethical Review at the University of Manitoba. If you have any concerns or complaints about this project, you may contact the above-named person or the Human Ethics Secretariat at 474-7122.

_____

Participant's Name (Please Print)

_____

Participant's Signature Date

Telephone Number _____E-mail Address _____

_____

Researcher and/or Delegate's Signature Date

I would like a copy of the purpose and the results of the study sent to the e-mail address noted above Yes _____ No _____

Panelist Number _____

Delegate's contact information:

Donna Ryland, Sensory Evaluation Specialist
Room 400 Human Ecology Building
Telephone - 474-8071
E-mail - ryland@cc.umanitoba.ca

Appendix F

Questionnaire – Bison Steak

This information will be kept strictly confidential.

Panelist # ____

1. Have you participated on sensory evaluation panels before?
Yes____ No ____ If yes,

a) What product(s) did you evaluate?

_____

_____

b) Was training part of the evaluation procedure? Yes ____ No ____
If yes, indicate for which product(s).

_____

_____

2. Are you allergic to any food products? Yes ____ No ____
If yes, note them below.

_____

_____

3. Are there any foods specifically, or food flavors and textures generally, that you would prefer not to evaluate?

_____

_____

Thank you very much for completing this questionnaire.

Appendix G

Sensory attribute definitions, method for evaluation, standard preparation and manufacturer for bison sensory evaluation

Aroma Attributes				
Attribute	**Definition**	**Method for evaluation**	**Standard preparation[1]**	**Standard manufacturer**
Beef	Aroma associated with stewed beef	Place the sample container in position for sniffing. Remove the cover. Take three short sniffs and replace the cover.	Top round beef[2] roast ~ 500 ± 20 g simmered in 1250 ml water in 9.5 L pot about 2.5 hours; pieces cut after cooking to 1.5 x 1.5 x 2 .5 cm	Safeway Inc,. local supermarket, Winnipeg, MB
Vinegar/Sour	Aroma associated with vinegar	Place the sample container in position for sniffing. Remove the cover. Take three short sniffs and replace the cover.	White vinegar, 4-5% acetic acid (30 ml amber vial with 3 drops vinegar on cotton ball)	H. J. Heinz Company of Canada Ltd., North York, ON M2N 7K5
Brothy/Salty	Aroma associated with beef broth powder	Place the sample container in position for sniffing. Remove the cover. Take three short sniffs and replace the cover.	Beef OXO powder (4.5 g) dissolved in boiling water (175 g) (according to directions), let stand approximately 20 minutes before serving; ~ 20 ml	Knorr, Unilever Canada, Toronto, ON M4W 3R2
Oily/Fatty	Aroma associated with cooked ground beef	Place the sample container in position for sniffing. Remove the cover. Take three short sniffs and replace the cover.	Regular (30% fat) ground beef (5.75 ± 0.25 g) cooked in foil dishes with foil lid in preheated oven (120°C) to 80°C held at 50°C on corning heater	Safeway Inc,. local supermarket, Winnipeg, MB

[1]Served in 60 mL plastic portion cups with lids unless otherwise noted

[2]Canadian AAA Grade

Appendix G

Sensory attribute definitions, method for evaluation, standard preparation and manufacturer for bison sensory evaluation

Flavour Attributes				
Attribute	**Definition**	**Method for evaluation**	**Standard preparation[1]**	**Standard manufacturer**
Beef	Flavor associated with broiled rib eye steak	Place one piece of sample in your mouth. Chew the sample thoroughly. Evaluate the flavor attribute intensities just before swallowing the sample.	Rib eye beef steak broiled at 550ºF to internal temperature 75ºC; pieces cut to 1.5 x 1.5 x 2.5 cm after cooking in foil dishes held at 50°C on corning heater	Safeway Inc,. local supermarket, Winnipeg, MB
Sour	Flavor associated with citric acid in water	Place one piece of sample in your mouth. Chew the sample thoroughly. Evaluate the flavor attribute intensities just before swallowing the sample.	0.01% (wt/vol) citric acid (0.01 g citric acid in 100 ml filtered water)	Rougier Pharma, Division of Ratiopharm Inc. Mirabel PQ Canada J7J 1P3
Salty	Flavor associated with sodium chloride in water	Place one piece of sample in your mouth. Chew the sample thoroughly. Evaluate the flavor attribute intensities just before swallowing the sample.	0.05% (wt/vol) sodium chloride (0.05 g sodium chloride in 100 ml filtered water)	Coarse salt; Sifto Canada Inc. Mississauga, ON L5N 6A4
Oily/Fatty	Flavor associated with cooked ground beef	Place one piece of sample in your mouth. Chew the sample thoroughly. Evaluate the flavor attribute intensities just before swallowing the sample.	Regular (30% fat) ground beef (5.75 ± 0.25 g) cooked in preheated oven (120°C) to 80°C held at 50°C on corning heater	Safeway Inc,. local supermarket, Winnipeg, MB

[1]Served in 60 mL plastic portion cups with lids unless otherwise noted

[2]Canadian AAA Grade

Appendix G

Sensory attribute definitions, method for evaluation, standard preparation and manufacturer for bison sensory evaluation

Texture				
Attribute	**Definition**	**Method for evaluation**	**Standard preparation[1]**	**Standard manufacturer**
Initial tenderness	The ease of biting through the sample across the grain of the meat with the molars	Take the piece of meat with the fork and place it so that the two ends of the sample are between your molars. Take one bite. Evaluate the ease of biting through the sample.	Add all beef wiener to boiling water, boil for 5 minutes to 75°C internal temperature, piece size about 1.5 x 1.5 x 1.5 cm served warm ~40-50°C	Schneiders, Kitchener, ON N2G 3X8
Juiciness	The amount of moisture present in the sample.	Chew the sample 2 times. Evaluate the amount of moisture present in the sample.	Bamboo shoot sliced, canned in water - piece size about 0.5 x 1.5 x 2.0 cm; served at room temperature (22°C)	Thai World Import and Export, 2532 Troknokket Ratchadaphisek Rd, Bangklo, Bangkholaem, Bangkok Thailand 10120
Overall tenderness	The ease of chewing the sample	Chew the sample 4 to 5 times. Evaluate the ease of chewing the sample.	Mozzarella cheese - piece size about 1.5 x 1.5 x 1.5 cm; served at refrigerator temperature (4°C)	Kraft Canada Inc., Don Mills, ON, M3B 3L6
Connective tissue	The amount of connective tissue present in the sample.	Chew the sample 4 to 5 times. Evaluate the amount of connective tissue present in the sample.	Inside round beef steak cut into 1.5 x 1.5 x 2.5 cm pieces, boiled for 5 minutes to internal temperature of 85ºC, served warm ~40-50°C	Safeway Inc,. local supermarket, Winnipeg, MB

[1]Served in 60 mL plastic portion cups with lids unless otherwise noted

[2]Canadian AAA Grade

Appendix G

Sensory attribute definitions, method for evaluation, standard preparation and manufacturer for bison sensory evaluation

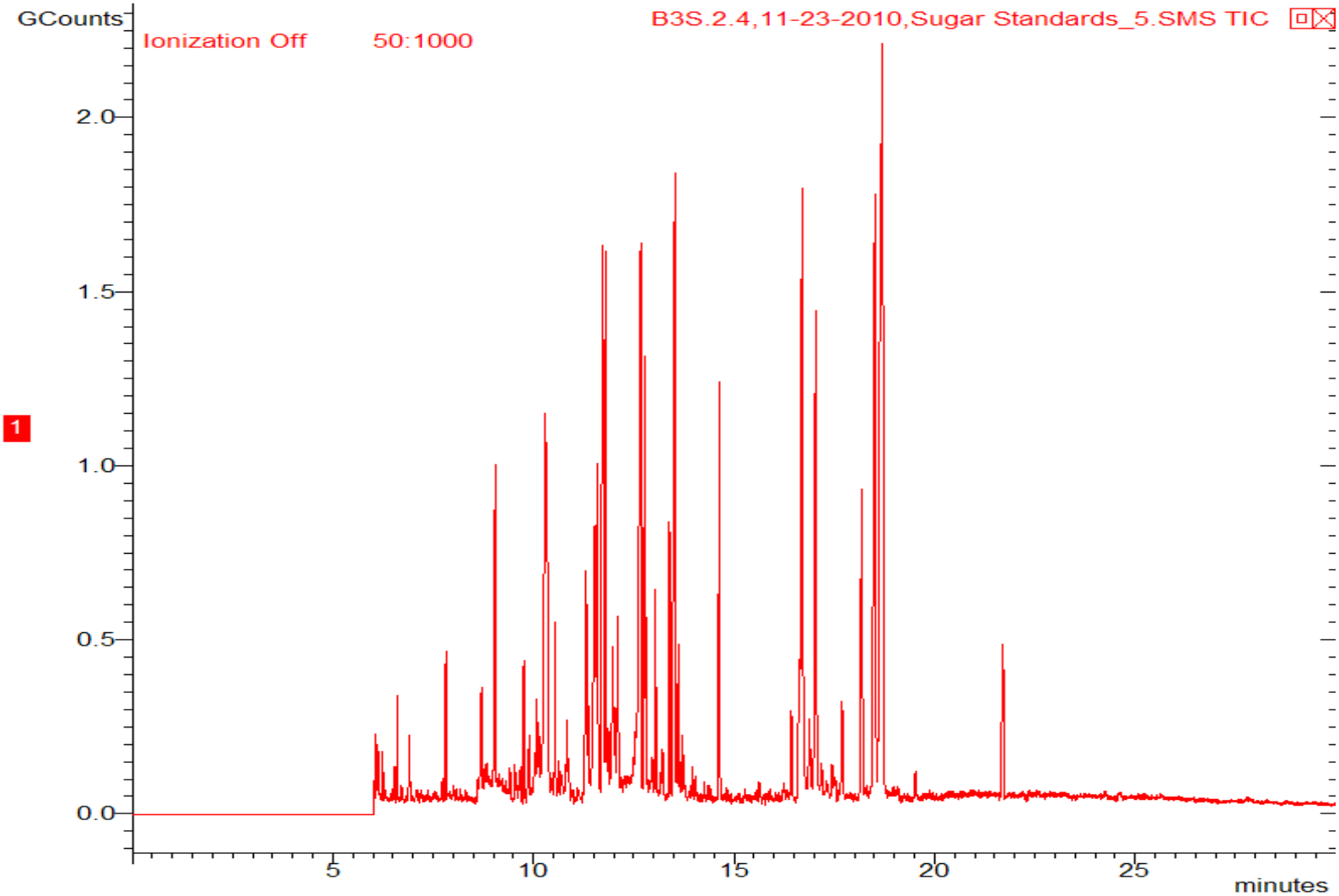
Texture				
Attribute	**Definition**	**Method for evaluation**	**Standard preparation[1]**	**Standard manufacturer**
Chewiness	The length of time required to prepare the sample for swallowing.	Chew the sample completely. Evaluate the length of time required to prepare the sample for swallowing.	Nibs licorice (cherry flavor) - one piece (approximately 1.5 cm long and 1 cm diameter (1.5 g)) served at room temperature (22°C)	Hershey Canada Inc. Mississauga, ON L4W 0B1
Oily mouth coating	The amount of oily film remaining in the mouth after swallowing.	Chew the sample completely. Evaluate the amount of oily film remaining in the mouth after swallowing.	Regular (30%) ground beef (5.75 ± 0.25 g) cooked in foil dishes with foil lid in preheated oven (120°C) to 80°C held at 50°C on corning heater	Safeway Inc,. local supermarket, Winnipeg, MB

[1]Served in 60 mL plastic portion cups with lids unless otherwise noted

[2]Canadian AAA Grade

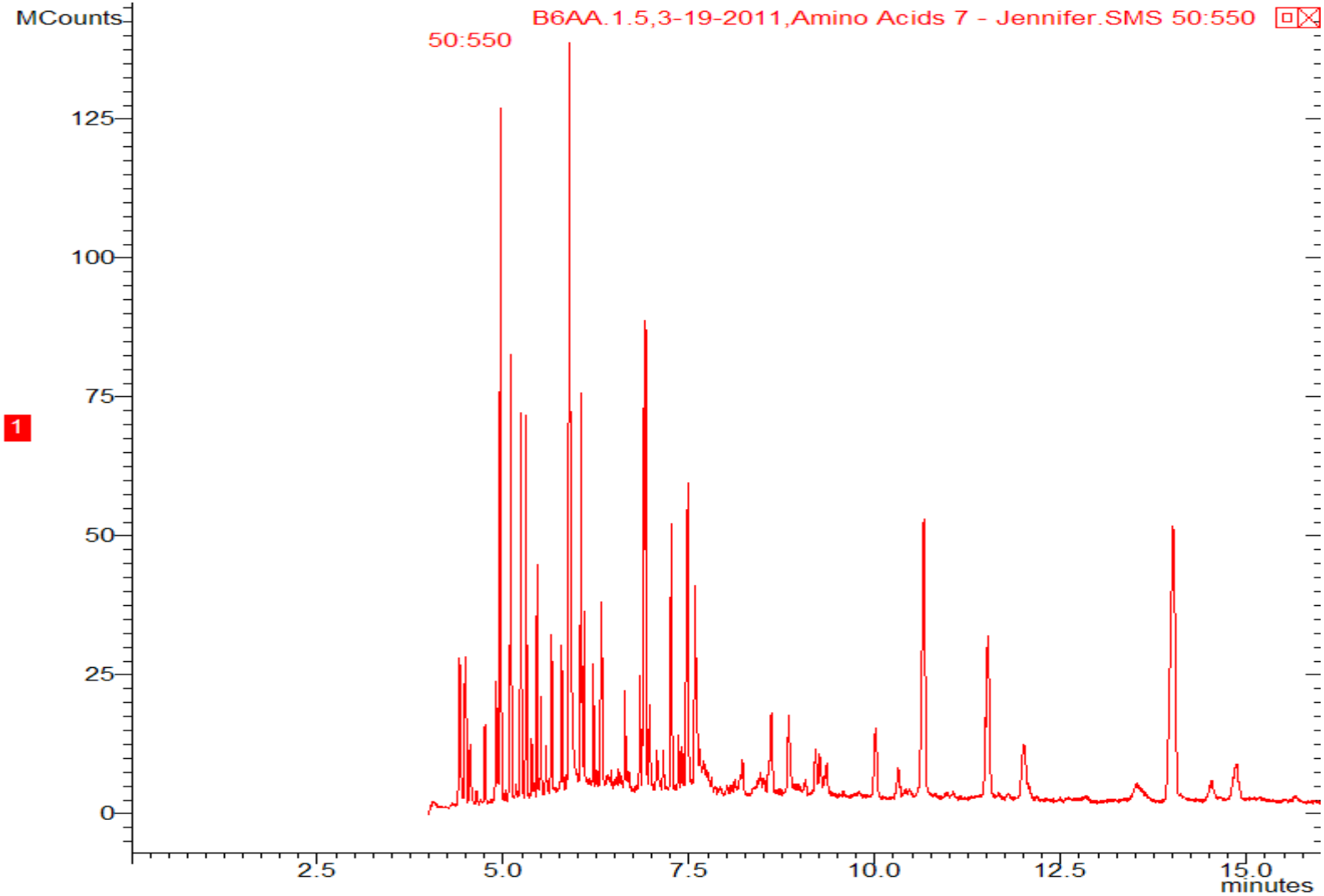
Appendix H

Example of Bison Chromatogram – Reducing Sugars & Sugar Phosphates



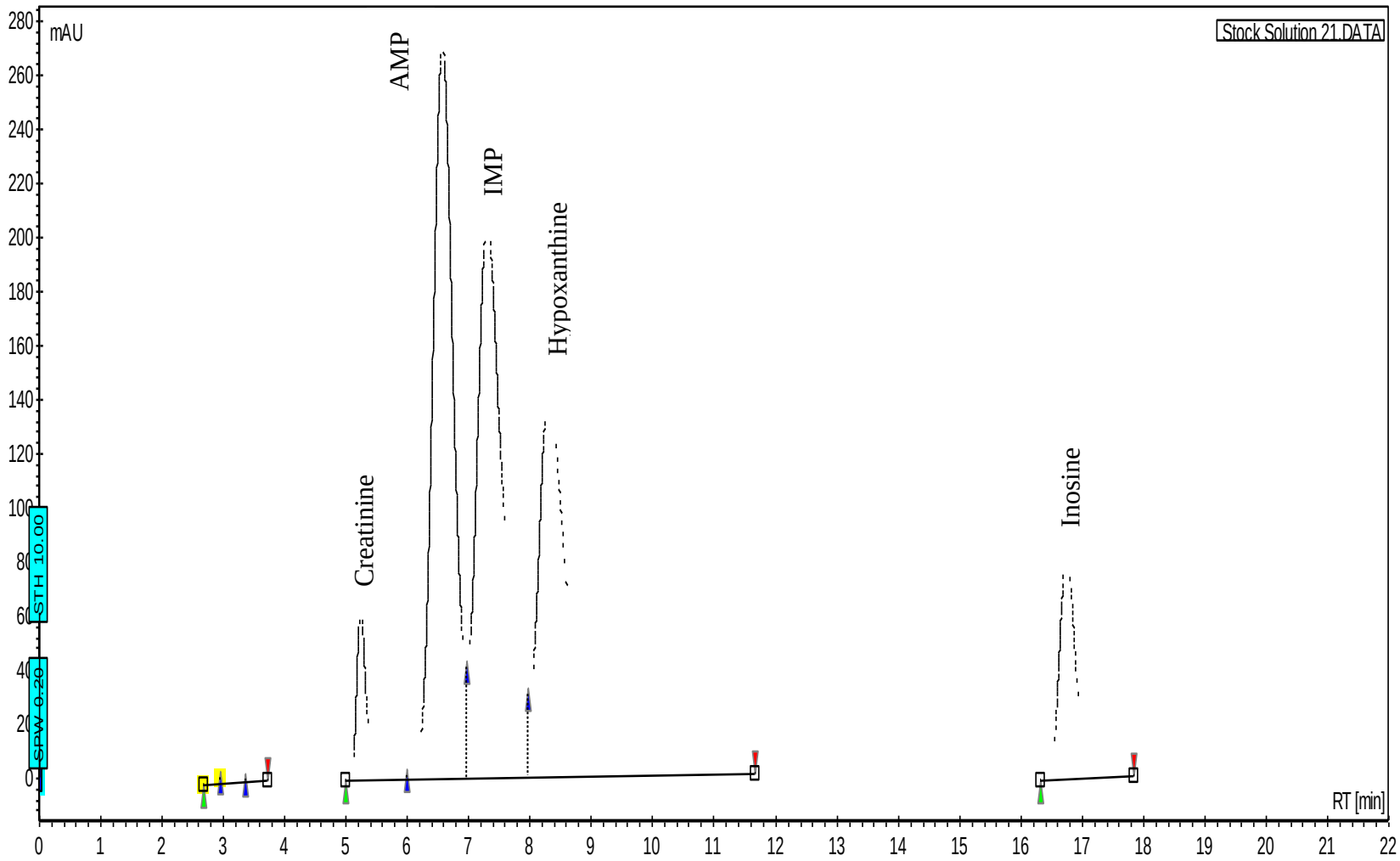
Appendix I

Example of Bison Chromatogram - Amino Acids



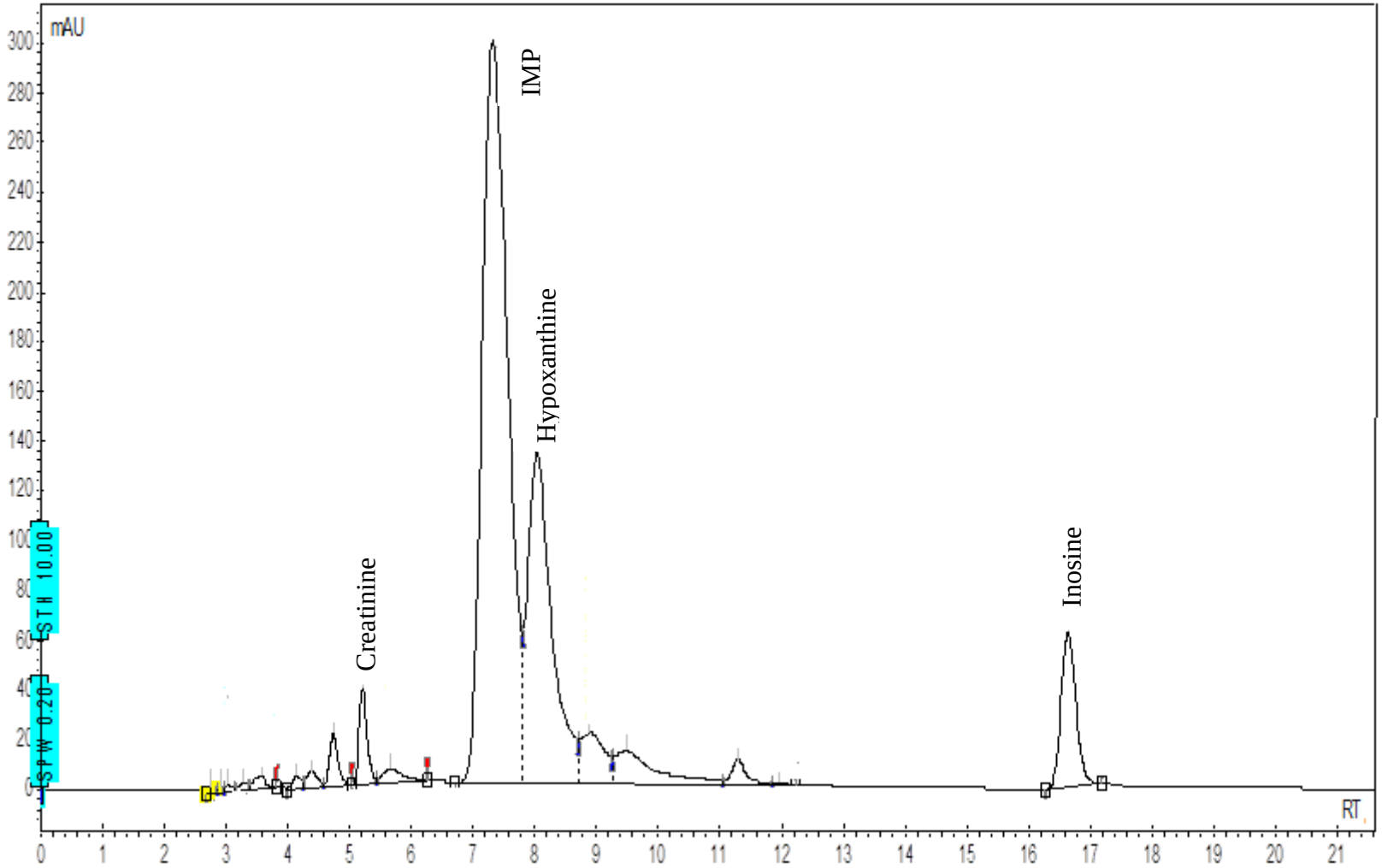
Appendix J

Example of Standard Solution Chromatogram – Nucleotides, Nucleosides and Bases



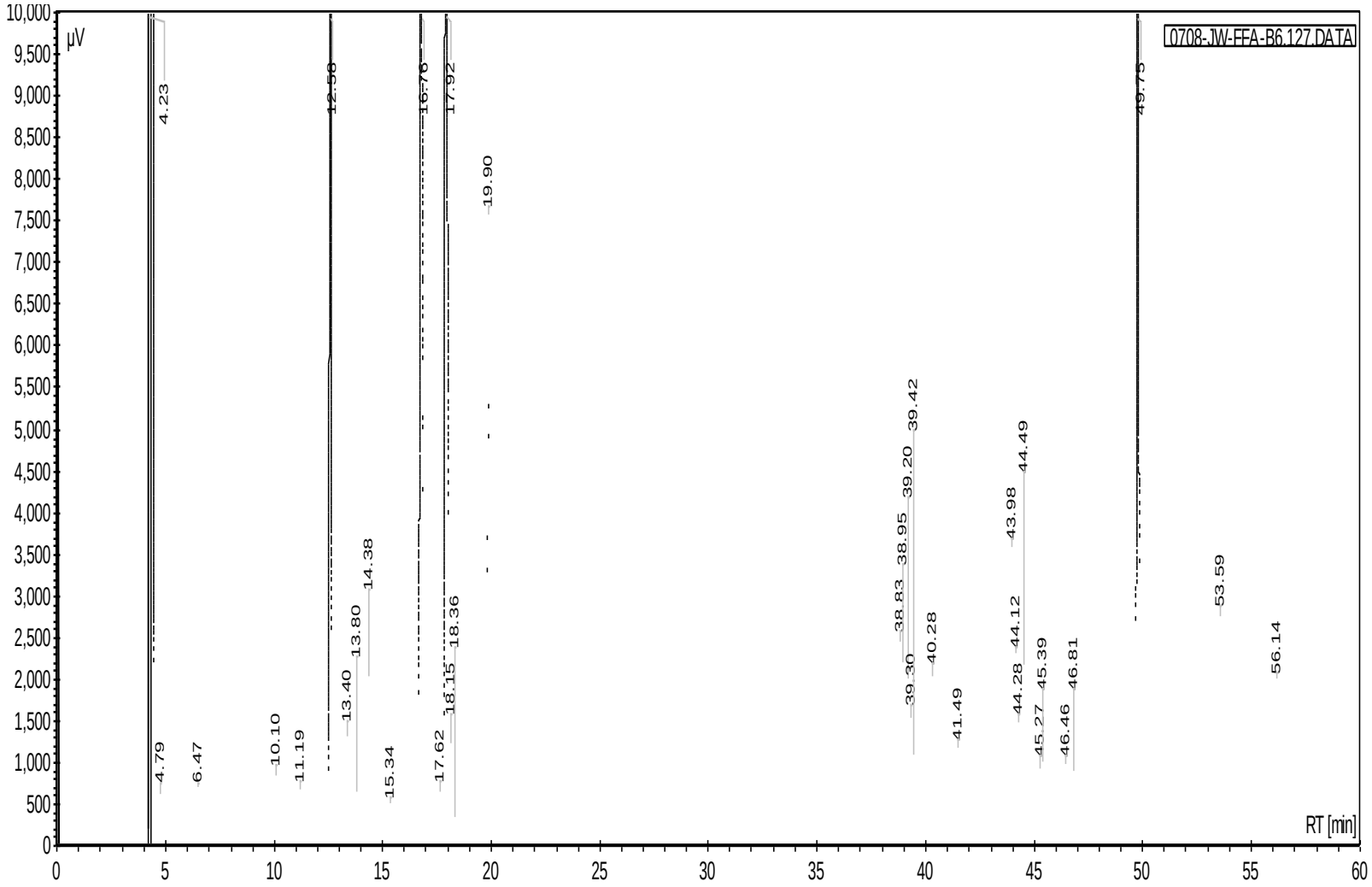
Appendix K

Example of Bison Chromatogram – Nucleotides, Nucleosides and Bases



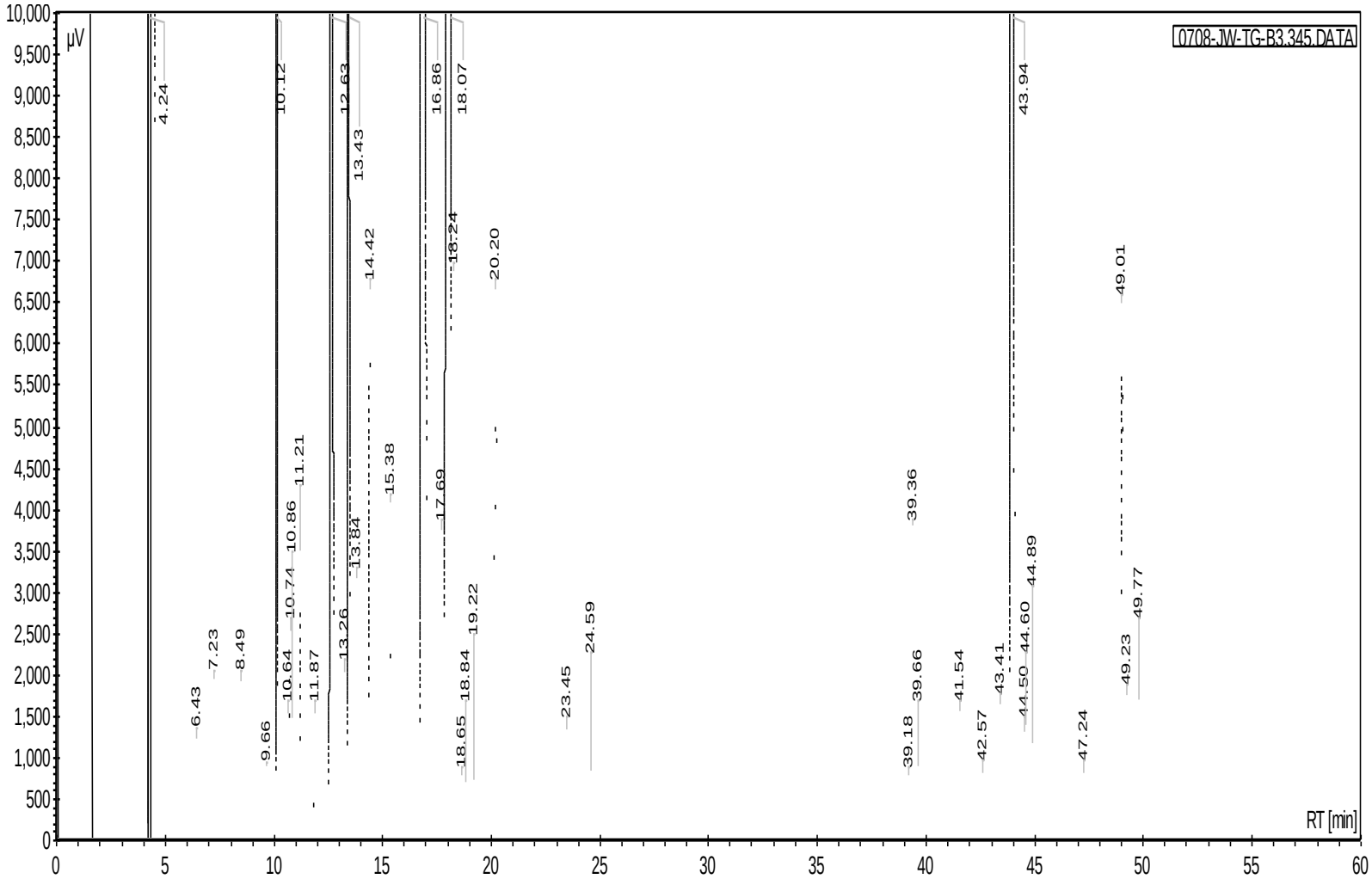
Appendix L

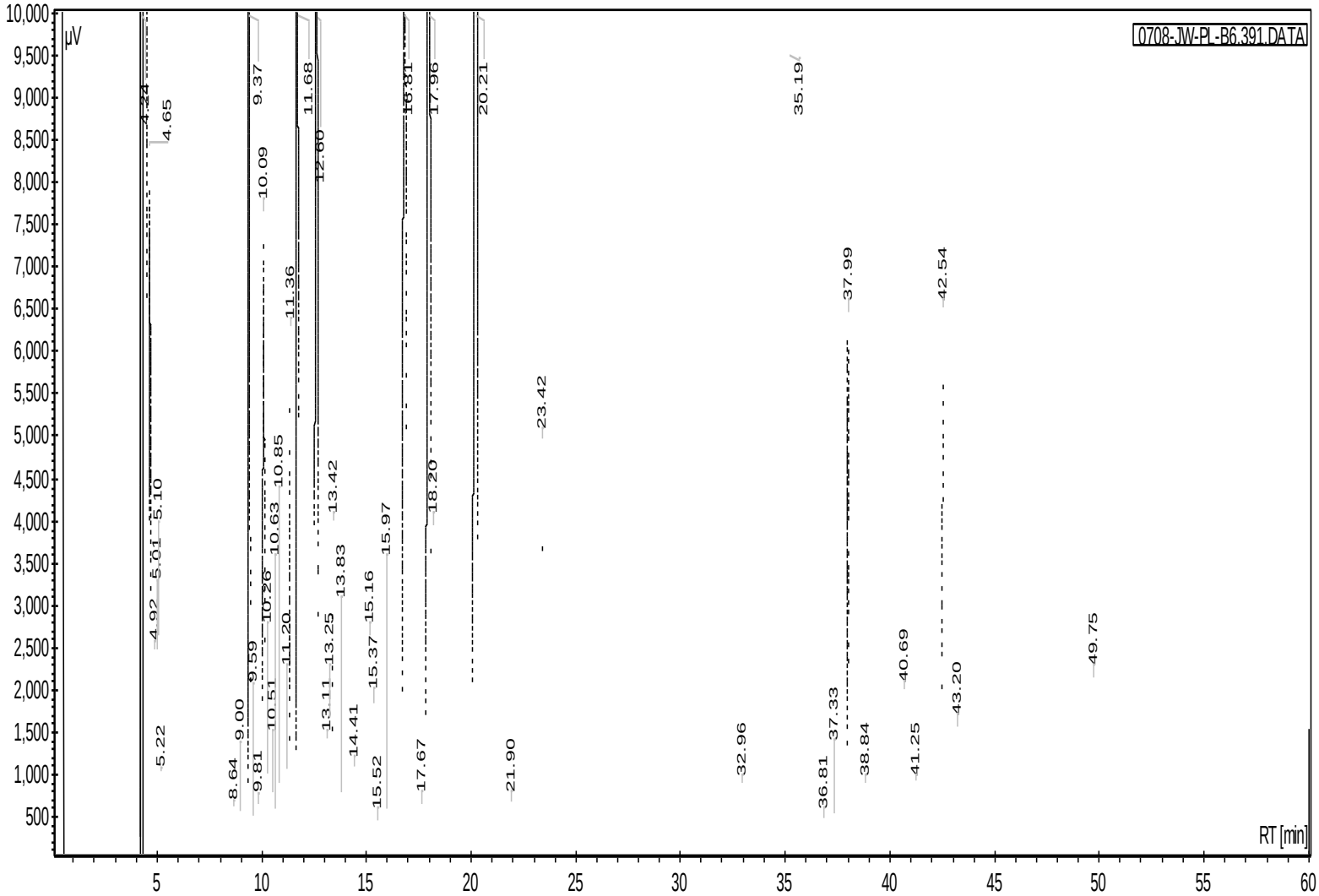
Example of Bison Chromatogram – Free Fatty Acids



Appendix M

Example of Bison Chromatogram - Triglycerides



Appendix N

Example of Bison Chromatogram – Phospholipids