

QUALITY ATTRIBUTES OF READY-TO-EAT BISON MEAT SNACKS
DURING 40°C ACCELERATED STORAGE

by

JAMES DANIEL HEITSCHMIDT

B.S., Kansas State University, 2012

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2015

Approved by:

Major Professor
Elizabeth A. E. Boyle

Copyright

JAMES HEITSCHMIDT

2015

Abstract

The market for bison meat products is increasing as a result of consumer interest in meat from animals that are primarily grass fed. Quality attributes of a bison meat snack containing cranberry and apple pieces and formed into a bar or bite were evaluated during 18 weeks of storage in a 40°C accelerated shelf life cabinet to simulate an ambient shelf life of 18 months. The products were formulated at a commercial facility; bars were packaged into a vacuum package, while bites were packaged in a sealed bag with an oxygen absorber. External color, pH, sensory attributes, Warner-Bratzler shear force (WBSF), water activity (a_w), and yeast and mold populations were determined. External color, pH, and a_w were evaluated on weeks 0, 3, 5, 12, 14, and 18 of accelerated storage. Sensory attributes, WBSF shear force, and yeast and mold populations were evaluated on weeks 0, 3, 5, 12, and 18. At week 0 and week 18, external L* for the bars and bites were similar ($P>0.05$); however, trained panelists observed both products becoming visually darker ($P<0.05$) by weeks 3 and 12 for bars and bites, respectively. For bars and bites, a^* values remained constant ($P>0.05$) through week 5 and 12, respectively, and then became less red ($P<0.05$) by week 14 for bars and bites. Bars continued to become less red ($P<0.05$) by week 18. Bar a_w remained constant ($P>0.05$) from week 0 through week 18, while bites a_w remained constant through week 5, and then declined ($P<0.05$) to a mean a_w of 0.83 by week 18. Bar pH remained constant ($P>0.05$) through week 5, and then declined ($P<0.05$) to 4.32 at week 18. Bites pH declined ($P<0.05$) from 4.63 at week 0 to 4.22 at week 18. Yeast and mold populations were non-detectable throughout storage for both products. Panelists found that bar bite and tenderness remained similar ($P>0.05$) from 4.63 at week 0 through week 18. Bites became softer and more tender ($P<0.05$) from week 0 to week 3, and then remained similar ($P>0.05$) through week 18. Bars and bites WBSF remained similar ($P>0.05$) from week 0 through week 18. Bar sweetness and fruit flavor intensity declined ($P<0.05$) and bar and bite off-flavors increased ($P<0.05$) by the end of storage. Changing product size from bars to bites and using a vacuum bag versus a sealed bag with an oxygen absorber influenced product characteristics during accelerated storage. The recommended shelf life for bars and bites would be equivalent to 5 months at ambient temperature based on 5 weeks at accelerated storage at 40°C.

Table of Contents

List of Tables	vi
Chapter 1 - Literature Review.....	1
Shelf Life Determination	1
Accelerated Shelf Life	1
Intermediate Moisture Meats	4
Packaging.....	6
Moisture Migration.....	7
<i>Bison bison</i>	8
Bison Lipid Composition Compared to Cattle	10
Chapter 2 - Quality Attributes of Ready-to-Eat Bison Meat Snacks During 40°C Accelerated Storage	13
Introduction.....	13
Materials and Methods.....	14
Product Description.....	14
Study Design.....	14
Water Activity.....	15
Moisture and Protein Analysis.....	15
pH.....	15
Yeast and Mold	16
Sensory Analysis.....	16
Shear Force	17
Instrumental External Color.....	17
Statistical Analysis	17
Results and Discussion	18
Physicochemical Properties and Yeast and Mold Population for Bars.....	18
Physicochemical Properties and Yeast and Mold Populations for Bites	19
Sensory Attributes for Bars.....	20

External Color	20
Texture	21
Flavor Intensity	21
Sensory Attributes for Bites	23
External Color	23
Texture	23
Flavor Intensity	23
Instrumental Color for Bars	24
Instrumental Color for Bites	25
Conclusions	27
Bibliography	28
Appendix A - Sensory Evaluation Form.....	32
Appendix B - Statistical Code for Bars and Bites.....	33
Sensory Analysis.....	33
Physiochemical Analysis	34
Instrumental Color Analysis	36
Appendix C - Probability Tables	37

List of Tables

Table 2-1. Least squares means for physicochemical properties for ready-to-eat bison bars ¹ during accelerated storage at 40°C.	19
Table 2-2. Least squares means for physicochemical properties for ready-to-eat bison bites ¹ during accelerated storage at 40°C.	20
Table 2-3. Least squares means for sensory and instrumental attributes for ready-to-eat bison bars ¹ evaluated over 18 weeks of accelerated storage at 40°C.	22
Table 2-4. Least squares means for sensory attributes for ready-to-eat bison bites ¹ evaluated over 18 weeks of accelerated storage at 40°C.	24
Table 2-5. Least squares means for instrumental color for ready-to-eat bison bars ¹ evaluated over 18 weeks of accelerated storage at 40°C.	25
Table 2-6. Least squares means for instrumental color for ready-to-eat bison bites ¹ evaluated over 18 weeks of accelerated storage at 40°C.	26
Table C-1. Probability values for week of storage for physicochemical properties for ready to eat bison bars during accelerated storage at 40°C.	37
Table C-2. Probability values for week of storage for physicochemical properties for ready to eat bison bites during accelerated storage at 40°C.	38
Table C-3. Probability values for week of storage for sensory and instrumental attributes for ready to eat bison bars during accelerated storage at 40°C.	38
Table C-4. Probability values for week of storage for sensory and instrumental attributes for ready to eat bison bites during accelerated storage at 40°C.	39
Table C-5. Probability values for week of storage for instrumental color for ready to eat bison bars during 18 weeks of accelerated storage at 40°C.	39
Table C-6. Probability values for week of storage for instrumental color for ready to eat bison bites during 18 weeks of accelerated storage at 40°C.	40

Chapter 1 - Literature Review

Shelf Life Determination

Shelf life can be defined as the amount of time a product can be stored under defined storage conditions after being produced. The conclusion of shelf life is determined by the time point that the product fails, whether it be for food quality or food safety reasons. Determining the shelf life of food products is generally assessed using different shelf life procedures, one being accelerated storage. Accelerated shelf life testing is generally conducted to shorten testing time. Consumer acceptance should be taken into consideration when evaluating shelf life, and determining what thresholds consumers might find unacceptable (Labuza 1982a).

Oxygen (O_2) permeability of the packaging material plays a vital role in product quality during shelf life, also known as O_2 transmission rate. Depending on the product, O_2 may be necessary to enhance or maintain product quality; O_2 can also degrade vitamins present in food (Labuza 1982b).

Antioxidants are used to slow the rate of lipid oxidation (Labuza 1982b). The control of lipid oxidation is vital, as the reaction cannot be stopped, only controlled with the aid of packaging and/or formulation to reduce the effect of O_2 on the product (Coma 2006; Labuza 1982b). Oxidation of lipids can cause products to become rancid and therefore less desirable, and can even occur when products are in a frozen state (Labuza 1982b).

Moisture content is another attribute to consider when assessing product shelf life. Water vapor transmission rate in and out of a product, and the transmission rate through packaging will influence product characteristics (Labuza & Szybist 2001). Moisture migration within a product will occur, and can influence the water activity (a_w) of the product. With a heterogeneous product, the different water activities of each raw material must be considered. Product moisture will move from a high moisture area to a low moisture area within a product. Relative humidity within the headspace will influence microbial growth as well as yeast and mold growth (Labuza 1982a).

Accelerated Shelf Life

Accelerated shelf life testing is used to evaluate the shelf life of a product in a shorter time frame than storing a product at the normal storage temperature (Labuza 1982b). The main

advantage for selecting accelerated storage is the ability to obtain research data in a shorter amount of time. Product deterioration can be evaluated by assessing quality attributes and how they change or deteriorate over time to conclude failure. Product changes during accelerated shelf life may be “chemical, physical, biochemical, or microbial” (Mizrahi 2011). A number of different models can be used when assessing accelerated shelf life. One model is the Arrhenius model, which is a model that uses constant kinetic energy to make a linear model of product deterioration (Mizrahi 2011). To make calculations easier, temperature is increased by increments of 10°C to create a ratio, which is called Q_{10} (Mizrahi 2011). Different storage factors can be used to accelerate storage with temperature being the most commonly used factor.

Models can be used to predict the shelf life outcome of foods, and can be simple or complex. With some models, certain attributes should be known in order to use a particular model, such as water vapor and moisture content in foods (Labuza 1982b). Some products may not have a model that they fit into or the procedure used to evaluate the product may be too complex to be useful (Labuza 1982b). In addition, a deterioration index measures deterioration over time for food during storage. It takes into account the manipulation of different variables (Mizrahi 2011). A deterioration index is useful for sensory analysis, so the product can be determined as “being acceptable or unacceptable” (Mizrahi 2011). A rise in temperature can increase moisture loss of the product when compared to storage at lower temperatures (Okonkwo, Obanu & Ledward 1992b). Models can account for a multitude of different factors; however, Mizrahi (2011) stated that “1% of the extrapolated data might deviate from the real value by 99%.” Data accumulated from accelerated storage potentially can be misleading and this is why it is so important to use models or a deterioration index.

Okonkwo, Obanu, and Ledward (1992b) investigated stability of intermediate moisture smoked beef during storage under tropical conditions. They evaluated “moisture, water activity, solubility in SDS- β -mercaptoethanol solution, soluble hydroxyproline, available lysine, pH, haemopigment content, texture and fungi.” Beef *longissimus dorsi* was cooked in cups with three infused solutions formulated with sodium nitrite, sodium chloride, potassium sorbate, and with or without glycerol, and with or without an onion solution following Okonkwo, Obanu, and Ledward (1992a). In this study, samples were stored at 30 and 38°C for 12 weeks and moisture and a_w declined significantly for both storage temperatures. Samples stored at 38°C had greater decreases in moisture content and a_w than samples stored at 30°C. Sample pH steadily fell from

5.7 at week 0 to reach 5.3 by week 10, and then there was an increase in pH before falling again at week 12; the reason for an increase at week 10 was unclear. Samples peaked in shear force at weeks 8 and 10 and then fell at week 12. The increase in shear force was theorized to be due to the loss of moisture and protein cross linkage, which made the product brittle by the end of shelf life. Samples stored at 38°C had the most moisture loss as well as the highest shear force values (Okonkwo, Obanu & Ledward 1992b). Storing product at increased storage temperatures can increase moisture loss creating a tough or brittle product. Product characteristics change during accelerated storage, which can hinder organoleptic evaluation of the products. Sensory characteristics have not been shown to change at linear rates as storage temperatures increase (Waletzko & Labuza 1976; Okonkwo, Obanu & Ledward 1992b). Sensory attributes can have a much more rapid change at higher storage temperatures than during ambient storage.

Corradini and Peleg (2007) investigated ways to evaluate accelerated shelf life data. Data for microbial growth came from a Weibullian decay model, which uses time and temperature as factors affecting microbial growth (Corradini & Peleg 2007). The microbial model used data for *Pseudomonas fluorescens*, *Candida sake*, and *Yersinia enterocolitica* collected from Tyrer *et al.* (2004). Microbial growth curves tended to be a sigmoidal curve as microorganisms entered the death phase (Corradini & Peleg 2007). Microbial growth contributes to product spoilage that can determine shelf life. Growth of microorganisms are of concern when considering samples for sensory evaluation. Corradini and Peleg (2007) explained deterioration by a change in kinetics. They stated that chemical deterioration models take a non-linear shape when graphed. Given that no two products are exactly alike, Corradini and Peleg (2007) conceded that there might be a difference from actual experimentation and what models predict. Model systems are; however, the only way to account for many different variables, and can give understanding to data sets (Corradini & Peleg 2007). Finding the most useful model for a unique product can be most beneficial.

Waletzko and Labuza (1976) evaluated increasing temperatures on intermediate moisture foods and their effects on deterioration. In this study, hennican, a product with a_w of 0.85 and consisting of peanuts, freeze-dried chicken, raisins, Skippy® peanut butter, honey, non-fat dry milk, and water was used (Waletzko & Labuza 1976). The product was blended with 100 ppm of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in addition to citric acid, ascorbic acid, and K-sorbate, and then cut into 1.7 x 1.7 x 0.6 cm pieces (Waletzko & Labuza

1976). Half of the product was sealed in oxygen scavenging film and stored at 25, 35, and 45°C and the other half was stored in open air under the same conditions (Waletzko & Labuza 1976). They found that non-enzymatic browning accelerated so quickly that the browning may not be fully representative of product color under normal storage conditions. They concluded that “acceleration for the brown pigment development factor is about 9-14 times comparing 25 to 45°C” (Waletzko & Labuza 1976).

In addition, Waletzko and Labuza (1976) wanted to correlate browning with toughening; however, browning of the product was more extensive than the toughening and there was no difference in toughening and packaging type. With an increase in storage temperature it was determined that deterioration of a heterogeneous food was non-linear. Finally, the prediction of a shorter shelf life with storage at 45°C was considered by Waletzko and Labuza (1976) to be “a built-in safety factor for the food processor.” Increasing storage temperature can have a detrimental impact on the exterior color of food. Though a deterioration of color can occur at increased temperatures, the use of a model and/or other parameters may provide a better conclusion of when product reaches failure.

Intermediate Moisture Meats

Intermediate moisture meats are products which have had their a_w lowered by either processing or ingredients or both, and do not need rehydration prior to consumption. Intermediate moisture meat products do not typically require refrigeration and can be shelf stable. They can have their moisture content lowered by adding ingredients to chemically bind the water in the product. The most common way of reducing a_w for intermediate moisture meats is the use of humectants and salt (Labuza, Acott & Sloan 1974). Reducing a_w is important to inhibit growth of pathogens; however, mold growth can still occur at less than 0.85 a_w . Due to the fact that mold can still be active in intermediate moisture meat products, antimycotics can be added to inhibit mold growth (Labuza, Acott & Sloan 1974).

Obanu, Ledward, and Lawrie (1975b) researched intermediate moisture foods and examined deterioration mechanisms and quality for human nutrition. They cut beef *longissimus dorsi* into 1 cm cubed pieces, placed the cubes into cans and then infused them with solutions containing 9.5% NaCl, 0.5% antimycotic, and differing amounts of glycerol and water. Samples were then heated to an internal temperature of 70°C for 15 min. Cans were then tumbled at room

temperature for 16 h, then vacuum dried prior to storage at 38°C for 12 weeks (Obanu, Ledward & Lawrie 1975b). They found that pH did not change during storage; however, moisture decreased by 2.5% to 6% at week 12. Total plate counts declined by 2 logs in the first 3 weeks of storage. Mold populations increased from week 3 to week 6 before declining. They found no populations of halophiles or *Staphylococcus aureus* before or during storage. Soluble nitrogen (N) greatly increased during storage and packaging had no effect on solubility. It was concluded that increasing storage temperature increased moisture loss over time and decreased protein solubility (Obanu, Ledward & Lawrie 1975b). The loss of any free moisture would have an effect on microbial growth.

Webster, Ledward, and Lawrie (1980) evaluated the role of oxygen (O₂) on glycerol and salt in intermediate moisture meats stored at 38°C. Beef *longissimus* was prepared according to Obanu, Ledward, and Lawrie (1975b) with the samples being “immersed in 1.5 times their weight of infusing solutions containing NaCl (9.5%), antimycotic (0.5%), and differing amounts of glycerol and water.” Samples were cooked to 100°C for 15 min before being sealed. Some samples were sealed in bags. Samples were stored at 1, 17, and 38°C for 24 weeks for pH, N solubility in 3% SDS/1% β-mercaptopethanol, and hydroxyproline release determination (Webster, Ledward & Lawrie 1980). They found that pH of the samples stored at 17°C and 38°C fell from week 0 to week 6 from 5.89 to 5.17 and from 5.75 to 5.02, respectively. One sample stored at 38°C rose in pH between week 6 to week 15. Webster, Ledward, and Lawrie (1980) concluded that the rise and fall of pH occurred due to the breakdown of protein; that theory is supported by protein solubility data. They also stated that O₂ had a significant effect on increasing crosslink reactions, and found that anaerobic product crosslinking occurred minimally when compared to aerobic product. These results suggest that product storage temperature can create a tougher product when exposed to aerobic conditions.

Leonard and Larick (1990) compared changes in phospholipid fatty acid profile in cooked lean bison and Hereford meat due to frozen storage under vacuum packaging. They evaluated lipid content for neutral lipids, phospholipids, and conducted fatty acid analysis. Steaks for bison and Hereford were held for 6-8 months in frozen storage before being tempered at 20-22°C and trimmed into 5 mm cubes. Cubes were cooked to an internal temperature of 70°C before being packaged in an air-impermeable pouch and stored at -20°C for 28 days. They found that saturated fatty acids for bison and beef were similar with 49% and 52%, respectively,

for neutral lipids (NL). During vacuum-packaged frozen storage after cooking, unsaturated NL fatty acids degraded, suggesting that this fraction may contribute to off-flavors. Based on these results unsaturated fatty acid percentage can be affected by packaging type and storage conditions.

Formulating with ingredients that include reducing sugars can increase browning and lipid oxidation according to Labuza, Acott, and Sloan (1974). Also, lipid oxidation and non-enzymatic browning reactions are common with intermediate moisture foods (Labuza, Acott & Sloan 1974). Intermediate moisture meats can exhibit undesirable sensory characteristics depending on storage conditions, and with an increase in storage temperature, reaction rates will increase (Sahoo & Yadav 1994). They noted that with an increase in storage temperature, product shelf life might be reduced. Storage time may be extended when meat is not exposed to abusive extrinsic factors. Intermediate moisture meats are most readily used where refrigeration is limited. Hurdle technology can be a strategy to minimize microbial growth in intermediate moisture meats. Combining processing and formulation technology may lead to overall safety as treatments, ingredients, and packaging can improve safety (Chawla & Chander 2003).

Packaging

Different types of packaging along with films can affect product shelf life. Oxygen permeability is the most crucial aspect of packaging, as O₂ can be beneficial or detrimental to final product characteristics. The primary goal for packaging is to keep the product safe for the consumer. Different packaging methods can lead to different final characteristics for the same product. Packaging can have different intended uses such as cook-in-bag products. Films can be O₂ absorbing so that the packaging can modify the air in the headspace (Argyri, Panagou & Nychas 2012). Modified atmosphere packaging (MAP) is characterized by gas flushing of a package. By manipulating the O₂, N₂, carbon monoxide (CO), and carbon dioxide (CO₂) content, product color may be enhanced and/or extended (O'Sullivan & Kerry 2012). Cook-in packaging is another type of packaging that keeps a product safe and palatable for consumers. Other packaging films are designed to withstand pasteurization as well as high pressure processing (Argyri, Panagou & Nychas 2012). Packaging is crucial to maintain product color as well as keep food safe and presentable to the consumer. Vacuum packaging is a common type of

reduced O₂ packaging. Nitrogen is one gas used to displace O₂ and help to slow microbial growth and lipid oxidation in MAP. Nitrogen has no direct affect on meat color and is commonly used to fill or flush product packaging (Sebranek & Houser 2006). Carbon monoxide has been used in fresh meat packaging to obtain carboxymyoglobin (Sebranek & Houser 2006; Sorheim 2006). Carbon monoxide has a stronger affinity for myoglobin than oxygen, creating a much more stable fresh meat color (Sebranek & Houser 2006; Sorheim 2006). The incorporation of CO in packaging can extend product color shelf life in a refrigerated case (Sebranek & Houser 2006). Carbon monoxide has little effect on microbial growth and can influence consumer-buying decisions since consumers base freshness on color. It has been noted that product can spoil due to normal microbial growth, yet maintain fresh meat color (Sebranek & Houser 2006; Sorheim 2006). Carbon dioxide, another gas used in MAP, functions as an antimicrobial in packaging of meat, although CO₂ can have a negative effect on product pH (Sebranek & Houser 2006). Carbon dioxide is most useful when combined with other gases to maximize antimicrobial effects as well as preserving fresh meat color. Carbon dioxide does not have the same affinity for myoglobin when compared to CO; however, CO₂ can be absorbed into the meat itself, causing poor appearance to the consumer (Sebranek & Houser 2006).

Along with controlling the transfer of O₂ through film, water vapor transmission is another concern in MAP. Film barriers being used in packaging can be an important part of moisture control (Esse & Sarri 2004). Intended shelf life should be considered as packaging type influences final product characteristics. Microbial growth can change with a change in product headspace.

Moisture Migration

Moisture movement into a food product can negatively affect sensory attributes during product shelf life. Moisture will move from a high moisture area to a low moisture area. For example, if the relative humidity in the headspace of a package is high, then the product can absorb the moisture over time. Large ingredient pieces will exchange moisture at the points of contact within a product (Roudaut & Debeaufort 2011). Depending on the type of product, different laws and equations can be applied to determine the flow of moisture. Ficks law is an example of how porosity has an effect on moisture migration. According to Roudaut and Debeaufort (2011), there are two theories for moisture diffusion. One is that moisture diffusion

has uncoordinated molecular motion, otherwise called self-diffusion, which occurs on a molecular scale. The other theory is still molecular and has “chemical potential, often referred to as flow or bulk diffusion” and often occurs “through packaging films” (Roudaut & Debeaufort 2011). Two types of moisture movement can occur in a package at the same time. One type can occur in or on the product, where moisture is moving from a high a_w area to a low a_w area on a molecular scale. The second form involves moisture movement through the packaging and affects the headspace, which can subsequently affect the product.

Labuza (1982a) noted that moisture loss or gain in a product is regulated by law to protect consumers. Moisture variations within a product can have effects on safety and quality. A change in a_w can provide ideal conditions for pathogen growth post packaging. Esse and Sarri (2004) specified that without proper moisture control during storage, jerky style products could have an a_w reduction of 0.12 in a year. Water activity in a jerky with a shelf life over a year can be hard to maintain depending on packaging. An important consideration for keeping a product at its proper a_w is to inhibit mold and pathogen growth (Esse & Sarri 2004). Other considerations include whether a product could gain or lose moisture by the end of shelf life. Also, processors need to decide if product shelf life will be determined by sensory failure or by food safety concerns.

In the past, pemmican has been a staple food in Native American and Eskimo diets; pemmican is similar to jerky and is traditionally a high fat type meat product. Pemmican is unique because fruit is typically added to the meat to flavor and preserve it. In the past pemmican has also been a staple for many artic explorers because of its caloric density and shelf stability. Pemmican has mostly been consumed in a frigid climate, which slows lipid oxidation, helping to preserve the meat product. Pemmican traditionally was sun dried and covered in liquefied fat. Adding liquefied fat to the outside of the product reduced the a_w which reduced microbial growth. Just like other meat preservation techniques used by Native Americans, the process of sun drying was used to help preserve meat by dehydration. Fruit was also commonly added to pemmican to acidify and to add flavor (Thomas 1975).

Bison bison

In the past, bison have been a staple food source for Native American plains Indians (Barsh & Marlor 2003). Bison are able to withstand some of the harshest weather in North

America. One goal of ranchers has been to combine survivability of bison with the meat quality of *Bos taurus* (Cronin *et al.* 2013). Bison have genetically been crossbred with cattle to try and create a hybrid; however, the offspring presented no hybrid vigor which is why the two species are not typically crossbred. Bison were also crossed to try and help preserve the species, as bison were hunted to near extinction. Bison today still share some genetic traits with cattle (Cronin *et al.* 2013). Today, the National Bison Association (2014) strictly prohibits any cross breeding with cattle to maintain genetic purity of American bison herds. The majority of bison today are a part of private herds (Freese *et al.* 2006). Bison have the ability to graze and survive with little to no management in habitats that could be harsh for cattle.

Koch *et al.* (1995) evaluated for distinct differences between *Bos taurus* and *Bison bison* and a cross between the two species. They designed three experiments using Hereford and Charolais calves that were crossed with bison, which originated from Fort Niobrara Wildlife Refuge, Valentine, Nebraska. In the first experiment, animals were evaluated for growth, carcass characteristics, muscle biology, shear force, and sensory analysis. The second experiment consisted of using cattle that were of mixed breeds, a cross between Charolais and bison, and purebred bison. Feeding efficiencies were evaluated along with growth and carcass characteristics. The third experiment used cattle that were of mixed breeds, a cross between Charolais and bison, and purebred bison. Animals were evaluated for growth and digestion.

During the first experiment, cattle out-performed bison in growth during early stages of the experiment, but later in the experiment bison growth out-performed cattle. Poor growth by the bison early in the experiment was concluded to be due to the animals adapting to confinement. By the end of experiment one there were no differences for growth between Charolais and bison. Koch *et al.* (1995) found that bison had to become familiar with a head gate system for feeding as well as becoming familiar with eating a concentrate diet instead of grass. They also found bison ate less and grew slower when compared to cattle; however, bison had better feed conversion and were more efficient when compared to cattle in the first experiment. When trained panelists evaluated beef *longissimus* and bison *longissimus*, they found bison to have “a more intense ammonia, metallic and gamey flavor” (Koch *et al.* 1995). Fatty acid content for bison was higher in “unsaturated and polyunsaturated fatty acid composition” compared to beef (Koch *et al.* 1995). In the second experiment the cattle overall out-performed crossbred bison. During the third experiment cattle out-performed bison early in

the experiment; however, cold weather became a factor later in the experiment and bison outperformed cattle resulting in no difference overall. They concluded that a long adjustment period should be used for bison before starting any experiments because confinement pens and intake of a diet with moderate or high density are abnormal for bison. “They are not domesticated animals” (Koch *et al.* 1995). Bison being fed a concentrate diet can still have an increased amount of off flavors when compared to beef. The fatty acid profile can have greater amounts of unsaturated and polyunsaturated fatty acids (PUFA) compared to beef.

The bison industry can benefit state economies and add diversity and supply to niche markets. The objective of a study done by Sell, Bangsund, and Leistritz (2001) was to estimate the contribution from the bison industry to the economy of North Dakota. They evaluated economic data collected through surveys to evaluate the economic impact resulting from North Dakota’s bison industry. The bison industry mirrored the cattle industry in how producers tended to be either cow-calf or stocker finisher operations. They estimated that the bison industry directly affected the North Dakota economy by \$70 million annually, and can have a direct impact on the North Dakota economy. Raising bison continues to be a growing industry and can contribute a significant portion to North Dakota’s economy (Sell, Bangsund & Leistritz 2001).

Bison Lipid Composition Compared to Cattle

Distinct flavor and eating experiences occur between bison and beef. Diet can affect the lipid makeup of bison and cattle. Studies by Koch *et al.* (1995) and Rule *et al.* (2002) found that even when similar diets were fed to cattle and bison, bison still had significant increases in unsaturated fatty acids. Feeding cattle on grass is known to increase PUFA. Bison can have higher PUFA levels than beef, making bison meat more prone to a greater amount of oxidative flavors than beef (Koch *et al.* 1995).

Larick *et al.* (1989) evaluated phospholipid composition, fatty acid composition, and sensory analysis differences between bison and cattle. They used bison and Hereford steers that were finished on a diet consisting of 66% corn silage, 22% corn, and 12% soybean-mineral supplement and were harvested at 18 months of age. They found that bison had higher amounts of PUFA as well as stearic and linoleic acids. A large portion of bison PUFA’s came from linoleic acid. Phospholipid content for bison was similar to Brahman (Larick *et al.* 1989). Bison

had a higher amount of phospholipids when compared to Hereford (Larick *et al.* 1989). They found sensory traits of “bison were more intense in ammonia, bitter, gamey, liverish, old, rotten, and sour flavor.” These results were also supported by Leonard and Larick (1990). Having higher amounts of phospholipid content and PUFA can contribute to oxidative flavors and rancidity. Bison were found to have a greater amount of off flavors when compared to beef, even when finishing diets were the same. Phospholipids and PUFA also contribute to warmed-over flavor in cooked meats.

Marchello *et al.* (1989) researched bison nutrient composition to create an adequate database for nutrient composition of bison cuts that would be representative of what consumers would purchase. Bison, beef, pork, and chicken were evaluated for proximate analysis, sensory analysis, cholesterol, energy, mineral composition, and fatty acids. Diets for bison, cattle, pork, and chicken were not controlled so this could be why some fatty acids were found to be similar between bison and beef. They found no difference in linoleic acid between bison and beef. Mineral composition of bison meat compared to beef was significantly higher in “K, Na, P, Ca, Cu, Fe, Mg, Zn, and Mn” (Marchello *et al.* 1989). There were differences in values of moisture, fat, and protein across different studies; however, it has been pointed out that these differences could be due to diet of the bison (Koch *et al.* 1995; Marchello *et al.* 1989; Marchello *et al.* 1998). Fat percentages in the *longissimus* can be similar between bison and for a beef carcass that grades standard (Marchello *et al.* 1989). Bison were found to have a significantly greater amount of PUFA when compared to beef. (Larick *et al.* 1989; Marchello *et al.* 1989).

A trained sensory panel found that bison had more distinctive flavors when compared to beef (Koch *et al.* 1995; Larick *et al.* 1989). Grass fed beef has been found to be similar in PUFA to bison fed on grass (Rule *et al.* 2002). The fatty acid make up of bison could be contributing to different flavor characteristics that set bison apart from beef. Along with having a greater amount of PUFA, bison also have a different phospholipid composition (Koch *et al.* 1995; Larick *et al.* 1989). Increased concentration of phospholipids and PUFA’s may contribute to why bison meat oxidizes faster than beef (Larick *et al.* 1989; Rule *et al.* 2002). Bison has gained popularity due to its perceived health benefits compared to other red meats (Marchello *et al.* 1998). Bison is advertised as being lower in fat compared to beef, and studies by Koch *et al.* (1995) and Marchello *et al.* (1989, 1998) support this as they found that raw *longissimus* contained 1.9% to

2.9% fat. Bison compared to beef are higher in mineral content and this factor can increase oxidation and break down of phospholipids and PUFA (Marchello *et al.* 1998).

Chapter 2 - Quality Attributes of Ready-to-Eat Bison Meat Snacks

During 40°C Accelerated Storage

Introduction

It is not always practical or feasible to evaluate the shelf life of foods that are shelf stable and have an intended shelf life of more than a year using normal storage conditions. Accelerated storage provides actionable shelf life data in less time compared to ambient storage (Labuza 1982b). Shelf life of products can be accelerated by different methods; the most commonly used is temperature. When using accelerated storage, models are a useful tool to interpret data and to form an accurate conclusion (Mizrahi 2011). When increasing the storage temperature of food, the product can deteriorate differently than at lower temperatures (Mizrahi 2011). Results from accelerated shelf life data can be skewed, predicting a shorter shelf life if accelerated temperatures are used compared to evaluating shelf life at intended storage conditions (Labuza 1982b).

In the past, bison have been a food staple for the American Plains Indians. One type of product unique to the Native Americans was pemmican. Pemmican consisted of dried bison, fruit, and liquefied fat (Thomas 1975). Pemmican was popular on artic excursions as it was a shelf stable, high caloric food (Thomas 1975). Pemmican is considered an intermediate moisture meat product as it does not need rehydration and its water activity (a_w) may not be low enough to be classified as jerky (Labuza *et al.* 1974).

Several studies have shown distinct difference's between characteristics of bison and cattle meat such as bison meat has greater amounts of unsaturated fatty acids compared to beef (Koch *et al.* 1995; Marchello 1989; Larick *et al.* 1989). Due to having greater amounts of unsaturated fatty acids, bison has stronger off flavors like livery, gamey, rotten, and sour (Koch *et al.* 1995; Marchello 1989; Larick *et al.* 1989). Bison is often advertised as being leaner; however, beef graded standard was found to have a similar fat percentage as bison (Marchello 1989).

The type of packaging used for a food product can influence product characteristics during shelf life. For example, a permeable package used for fresh meat allows oxygen to move through the package changing meat color during refrigerated storage. Cured meat products require packaging films that prevent oxygen transmission and/or modified package atmosphere to reduce oxygen in a package. Modified atmosphere packaging can take the form of vacuum

packaging or flushing the package with different gases. Modifying the atmosphere for a meat product can help to either preserve the product and/or to enhance consumer acceptability (Sebranek & Houser, 2006).

The objective of this study was to evaluate quality attributes during accelerated storage of commercial bison meat snacks stored in vacuum packaging and sealed non-gas flushed packaging.

Materials and Methods

Product Description

Two shelf stable bison meat snack products in the form of bars and bites were evaluated. Samples were commercially obtained (Native American Natural Foods, Kyle, SD) and shipped to Kansas State University. Both bars and bites were formulated with bison meat, dried cranberries (cranberries, sugar), dried apples, sea salt, encapsulated lactic acid, celery juice, black pepper, cane crystals, dehydrated orange peel, spices, cinnamon, onion, and garlic powder. Bars were produced as 2.5 x 10 cm bars weighing 28 g while bites were 2.5 x 1.5-2.5 cm pieces packaged in a 85 g package. Bars were obtained in a vacuum package and the bites were in a sealed bag containing an oxygen scavenger. Both packaging types had a viewing portal on the packaging. The commercial supplier did not provide specifications on film characteristics.

Study Design

Upon arrival, bars (10 bars in a box) and bites (5 packages in a box) were left in their display boxes and not exposed to light. Bars and bites were placed in an accelerated shelf life cabinet (Model I22NL, Percival, Perry, IA) set at 40°C. Relative humidity was not controlled. Bars and bites were evaluated on weeks 0, 3, 5, 12, 14, 18 of accelerated storage. External color, pH, and a_w were evaluated on weeks 0, 3, 5, 12, 14, and 18. Sensory attributes, Warner Bratzler shear force (WBSF) and yeast and mold populations were evaluated on weeks 0, 3, 5, 12, and 18. Moisture and protein content were determined on weeks 0, 12, and 18. Two replicates were used for both bars and bites.

For accelerated storage and shelf life determination, $Q_{10} = 2$, was used to calculate shelf life at ambient temperature. A temperature of 20°C was used as the standard for ambient storage, and a $Q_{10} = 1$ was equal to 30°C. At this temperature, 1 week of accelerated storage = 2

weeks of ambient storage. With a $Q_{10} = 2$, 1 week of accelerated storage = 4 weeks at ambient storage. For deterioration, a $Q_{10} = 2$ at a constant temperature was calculated and yeast and mold growth was used as the mode of failure according to Labuza (1982b).

Water Activity

An a_w meter (Model 4TE, Decagon Devices, Inc. Pullman, WA) was used to measure a_w . The meter was standardized with 0.760 a_w and 0.984 a_w standards (Decagon Devices, Inc. Pullman, WA) to within 0.003. Two samples were taken from each bar and bite package following the procedure from Harper, Getty, and Boyle (2010) for a_w determination. Then, apple and cranberry pieces were manually separated with a knife from the bites to determine the a_w on apple pieces and cranberry pieces.

Moisture and Protein Analysis

Bars and bites were taken from one package, each were manually chopped into small pieces using a knife and submerged into liquid nitrogen. The pieces were then pulverized and homogenized in a stainless steel blender (Model 33BL79, Waring Products, New Hartford, CT) and placed into labeled 680 g whirl pak[®] bags (Nasco, Fort Atkinson, WI). The samples were then stored in an ultra low freezer at -80°C before being analyzed for moisture and protein content. Moisture content was measured using a SMART system 5 (CEM Corp., NC) following AOAC procedures (AOAC Official Method PVM-1:2003 MEAT). Crude protein was analyzed using a LECO TruMac N (Model 630-300-300, LECO Corp., St. Joseph, MI) system to measure nitrogen content following AOAC Official Method 990.03. Samples were taken in duplicate from a different bag.

pH

Bar and bite pH were analyzed using a pH probe (Hanna Instruments, H199163, Woonsocket, RI) attached to a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA). Two separate packages were used to measure pH for bars and bites at each sample time. A meat pH probe was inserted into one side and then on the other side of the product to determine product pH. A two-point calibration at pH 4 and 7 using standardized buffers (Thermo Fisher Scientific Inc, Waltham MA), was completed prior to measuring product pH.

Yeast and Mold

Enumeration of yeast and molds colonies was conducted using 3MTM yeast and mold Petrifilm (3M, St. Paul, MN). Bar and bite packages were opened and 10 g was aseptically removed. Samples were combined with 90 mL of 0.1% BactoTM peptone (Franklin Lakes, NJ) in a filter bag (FILTRA-BAG, no. 01-002-57, Fisher Scientific, Pittsburg, PA) and stomached (Stomacher 400 Lab Blender, Seward Medical, London, UK) for one minute. Samples were then serial diluted, plated in duplicate, and placed in an incubator at 20-26°C for 72 hr prior to enumeration.

Sensory Analysis

A trained sensory panel consisting of a minimum of six panelists who were Kansas State University faculty and graduate students was used. All sensory panelists were screened according to the American Society for Testing and Materials (1981) guidelines. Panelists were orientated to characteristics of the bars and bites during four training sessions. Training was conducted around a table where panelists were able to discuss attributes and scales. Attributes evaluated were external lean color, bite, tenderness, sweetness, saltiness, fruit flavor, sourness, and off flavor (Appendix A). An 8-point scale was used and samples were ranked to the nearest 0.5 increment (1= extremely light, extremely soft, extremely tender, none, not salty, none, none, none, and none; 2 = very light, very soft very tender, trace of sweetness, trace of salt, trace of fruit flavor, traces of sour, practically none; 3= moderately light, moderately soft, moderately tender, slight sweetness, slight saltiness, slight fruit flavor, slight sour, traces; 4= slightly light, slightly soft, slightly tender, small sweetness, small saltiness, small fruit flavor, small sour, slightly off; 5= slightly dark, slightly hard, slightly tough, modest sweet, modestly salty, modestly intense fruit flavor, modestly sour, slightly intense; 6= Moderately dark, moderately hard, moderately tough, moderately sweet, moderately salty, moderately intense fruit flavor, moderately sour, moderately intense; 7= very dark, very hard, very tough, very sweet, very salty, very intense fruit flavor, very sour, very intense; 8 = extremely dark, extremely hard, extremely tough, extremely sweet, extremely salty, extreme fruit flavor, extremely sour, extremely intense).

After being removed from the accelerated shelf life cabinet, samples were allowed to return to room temperature for 3 hr prior to being prepared for evaluation. Two samples were taken from different packages. Bars were manually cut in half lengthwise and then into 1.9 cm

pieces. Bites were manually cut into 1.9 cm x 1.9 cm pieces. Two pieces were portioned into a labeled plastic cup, capped with a lid, and presented to panelists under fluorescent lighting. Panelists were given distilled filtered water and unsalted soda crackers to cleanse their palate between samples.

Shear Force

Warner Bratzler shear force (WBSF) was determined using an Instron (Model 4201, Instron corp., Canton MA) fitted with an Instron flat blade. All samples were held at room temperature for a minimum of 1 hr before analysis. Samples were taken from separate packages for bars and bites. Whole bar pieces were sheared three times. The three largest pieces of bites were selected from the package and each sheared once per piece. Data was recorded using Instron Bluehill® software (Instron corp., Norwood, MA).

Instrumental External Color

External color of bars and bites was measured in duplicate, and duplicates were obtained from separate package's. Bar packages were opened from the designated package opening and measurements were taken in three places on a bar, beginning on the end closest to the opening of the package, in the middle, and then on the end of the bar. The bar was then flipped and three measurements were taken on the other flat side of the bar. Bites were removed from the packaging and three bites were used for color analysis. Bites too small for measurement were not used and placed back into the packaging. Bites were measured twice on each side. Measurements were taken with a Hunter Lab MiniScan EZ (Model 4500L, Hunter Associates Laboratory Inc., Reston, VA) using illuminant A with a 10° observer and measuring CIE lightness (L*), redness (a*), and yellowness (b*). The MiniScan was standardized prior to each use with black and white tiles provided by Hunter Associates Laboratory, Inc.

Statistical Analysis

A completely randomized design for bars and bites was used. Two replications were used for bars and bites. Week was the treatment, with six levels for bars and bites. Replication was not included in the model as there was only one degree of freedom. ANOVA was performed using the PROC mixed procedure in SAS (SAS Inst. Inc., Cary, NC) with a P<0.05 level of significance (Appendix B).

Results and Discussion

Physicochemical Properties and Yeast and Mold Population for Bars

Probability values for the week of storage for physicochemical properties for bars during accelerated storage are summarized in Appendix C-1. Initial a_w was 0.84 at week 0 and remained similar ($P>0.05$) through week 18 with a final a_w of 0.77 (Table 2-1). Moisture content for bars decreased ($P<0.05$) from 38.6% at week 0 to 33.5% by week 12, and then remained similar ($P>0.05$) through week 18 to a final moisture content of 28.2% (Table 2-1). These findings do not agree with research by Okonkwo, Obanu, and Ledward (1992) found a_w decreased during storage for cooked beef stored in loosely wrapped bags. Okonkwo, Obanu, and Ledward (1992b) found that smoked beef stored at 38°C had greater decreases in moisture content and a_w than samples stored at 30°C. Moisture will move during storage, from a high level area to a low area level (Labuza 1982a). Whether moisture is gained or lost depends on packaging and packaging atmosphere as well as raw materials (Labuza 1982a). Protein content for bars remained similar ($P>0.05$) from week 0 through week 18 (Table 2-1). The moisture to protein ratio (MPR) for bars decreased ($P<0.05$) from 1.78 at week 0 to 1.46 at week 12, and then remained similar ($P>0.05$) from week 12 through week 18 to a final MPR of 1.19 (Table 2-1).

Initial bar pH was 4.59 at week 0, and remained similar ($P>0.05$) from week 0 to 4.63 at week 5 before decreasing ($P<0.05$) to 4.30 at week 12 (Table 2-1). Bar pH then remained similar ($P<0.05$) from 4.30 at week 12 to 4.32 at week 18 (Table 2-1). A canned product from a study by Obanu, Ledward, and Lawrie (1975b) showed no change in pH during storage. Webster, Ledward, and Lawrie (1982) found that beef processed to a_w of 0.85 and stored at 38°C had a pH decrease during storage; however, one sample decreased then increased in pH over time. Malik and Sharma (2014) found that the pH of buffalo meat stored at 30°C in vacuum and aerobic packaging significantly decreased during seven weeks of storage, and concluded that packaging had no effect on pH. Malik and Sharma (2014) concluded that sugars consumed by microbes contributed to the pH decline. Yeast and mold analysis of bars were <100 estimated cfu/g from week 0 through week 18. Findings by Obanu, Ledward, and Lawrie (1992a) showed mold populations increased with time. Malik and Sharma (2010) found that yeast and mold were higher in aerobic packaging; however populations remained below 3.0 logs during storage.

Table 2-1. Least squares means for physicochemical properties for ready-to-eat bison bars¹ during accelerated storage at 40°C.

Attributes	Week						SEM ²
	0	3	5	12	14	18	
Water Activity	0.84	0.83	0.83	0.82	0.82	0.77	0.02
Percent Moisture	38.6 ^a	-	-	33.5 ^b	-	28.2 ^b	1.51
Percent Protein	21.9	-	-	23.1	-	23.5	0.88
MPR ³	1.78 ^a	-	-	1.46 ^b	-	1.19 ^b	0.08
pH	4.59 ^a	4.58 ^a	4.63 ^a	4.30 ^b	4.31 ^b	4.32 ^b	0.09

^{a-b} Least squares means with different letters within rows are significantly different ($P<0.05$).

¹ n=2.

² ±Standard error of the mean.

³Moisture protein ratio = MPR (moisture % / protein %).

- = not analyzed.

Physicochemical Properties and Yeast and Mold Populations for Bites

Probability values for the week of storage for physicochemical properties for bites during accelerated storage are summarized in Appendix C-2. Initial bite a_w was 0.88 at week 0 and a_w remained similar ($P>0.05$) through week 5. Water activity remained constant ($P>0.05$) from week 5 to week 14, and then bite a_w decreased ($P<0.05$) from week 14 to week 18 ending with an a_w of 0.82 (Table 2-2). Water activity of the apple and cranberry pieces followed a similar pattern to bars, having a final a_w of 0.82 at week 18. Moisture content for bites declined ($P<0.05$) from 43.1% at week 0 to 35.5% at week 12 and then remained constant ($P>0.05$) at 35.0% at week 18. Moisture moved out of both the meat and the fruit, drying the bites over time. The loss of moisture is critical as the product will lose moisture at a faster rate at 40°C than what may actually occur, so the number of weeks that a_w was below 0.86 may be overstated (Labuza & Schmidl 1985). Protein content for bars increased ($P<0.05$) from 18.1% at week 0 to 20.9% at week 12 and then ended at 23.9% at week 18. Moisture protein ratio decreased ($P<0.05$) from 2.39 at week 12 to 1.48 at week 18.

Initial bite pH was 4.63 at week 0, and remained constant ($P>0.05$) to week 3. Bite pH then declined ($P<0.05$) to 4.22 by week 18 (Table 2-2). Yeast and mold analysis of bites were <100 estimated CFU/g from week 0 through week 18.

Table 2-2. Least squares means for physicochemical properties for ready-to-eat bison bites¹ during accelerated storage at 40°C.

Attributes	Week						SEM ²
	0	3	5	12	14	18	
Water Activity	0.88 ^a	0.87 ^{ab}	0.86 ^{abc}	0.84 ^c	0.85 ^c	0.82 ^d	0.01
Apples, a_w ³	0.88 ^a	0.86 ^{ab}	0.85 ^{bc}	0.84 ^{bc}	0.84 ^c	0.82 ^d	0.01
Cranberries, a_w ⁴	0.88 ^a	0.86 ^b	0.85 ^{bc}	0.84 ^c	0.84 ^c	0.82 ^d	0.01
Moisture, %	43.1 ^a	-	-	35.5 ^b	-	35.0 ^b	1.11
Protein, %	18.1 ^c	-	-	20.9 ^b	-	23.9 ^a	0.69
MPR ⁵	2.39 ^a	-	-	1.70 ^b	-	1.48 ^c	0.12
pH	4.63 ^a	4.59 ^{ab}	4.56 ^{bc}	4.42 ^{bc}	4.41 ^c	4.22 ^d	0.06

^{a-d} Least squares means with different letters within rows are significantly different ($P<0.05$).

¹ n=2.

²±Standard error of the mean.

³Water activity for apple pieces removed from bites.

⁴Water activity for cranberry pieces removed from bites.

⁵Moisture protein ratio = MPR (moisture % / protein %).

- = not analyzed.

Sensory Attributes for Bars

Probability values for the week of storage for sensory attributes for bars during accelerated storage are summarized in Appendix C-3.

External Color

Sensory panelists found that bars visually darkened ($P<0.05$) from 5.3 at week 0 to 5.9 at week 3. Bars then became darker ($P<0.05$) with a score of 6.7 at week 12, and then darkened ($P<0.05$) further to 7.6 at week 18 (Table 2-3). Product discoloration can occur at such a rapid rate during accelerated storage that the deterioration may not be indicative of normal storage (Labuza 1982b; Waletzko & Labuza, 1976). Darkening of bars can be due to non-enzymatic browning, which is between a reducing sugar and an amino group (Zhang *et al.* 1994). Non-enzymatic browning occurs at an accelerated rate in the intermediate moisture range (Labuza Acott & Sloan, 1974). Browning rate was found to peak at an a_w of 0.43, and as moisture content increased browning rate decreased (Labuza Acott & Sloan 1974). Furthermore, Labuza,

Acott, and Sloan (1974) found that reducing sugars accelerate browning as temperature is increased from 25 to 45°C. Labuza, Acott, and Sloan (1974) found that browning occurred at a slower rate when product was in oxygen free packaging. Non-enzymatic browning effects cannot be reversed and the rate will increase with time (Friedman 1996). Waletzko and Labuza (1976) supported these findings, as they found non-enzymatic browning to increase with time. Hennican stored in modified atmosphere packaging with an oxygen scavenger had a browning rate that was slower than samples stored in air (Waletzko & Labuza 1976). Malik and Sharma (2014) found that the appearance of RTE buffalo meat stored at 30°C in vacuum and aerobic packaging became less desirable during seven weeks of storage.

Texture

Sensory bite force and tenderness for bars remained similar ($P>0.05$) from week 0 to week 18 (Table 2-3). Bars had the highest WBSF at week 18 although WBSF was similar ($P>0.05$) during 18 weeks of storage (Table 2-3). Okonkwo, Obanu, and Ledward (1992a) found that beef *longissimus* samples, regardless of storage at 30 or 38°C or smoked, increased in toughness until weeks 8 and 10 before declining. A loss in moisture content was seen to increase toughness until week 12 where samples became brittle and shearing was easier (Okonkwo Obanu & Ledward 1992b). Waletzko and Labuza (1976) found that hennican samples stored at 45°C became tougher than product stored at 25°C. Waletzko and Labuza (1976) concluded that intermediate moisture foods should use low oxygen packaging to optimize shelf life. Malik and Sharma (2014) found that the texture of buffalo meat stored at 30°C in vacuum and aerobic packaging became more desirable at week one of storage; however, by the end of storage at seven weeks the texture became less desirable.

Flavor Intensity

Bar sweetness remained constant ($P>0.05$) with a score of 3.1 at week 0 and 2.7 at week 12, and then bars became less sweet ($P<0.05$) with a score of 2.1 by week 18. Saltiness increased ($P<0.05$) from weeks 0 through week 3. Saltiness remained similar ($P>0.05$) from week 3 through week 12, and then panelists perceived bars to be less salty ($P<0.05$) from week 12 until week 18 (Table 2-3). By week 18, saltiness was perceived to be similar ($P>0.05$) as it was on week 0. Fruit flavor remained similar ($P>0.05$) from week 0 to week 12, and was less

fruity ($P<0.05$) by week 18 than the first 5 weeks of storage. Sourness for bars remained similar ($P>0.05$) with a score of 2.2 at week 0 and a score of 2.8 at week 18 (Table 2-3). Panelists were unable to perceive changes in sourness while pH slightly declined over time (Table 2-1). Off flavor intensity for bars was similar ($P>0.05$) from week 0 through week 12, and then increased ($P<0.05$) by week 18 (Table 2-3). Bison have a greater amount of unsaturated fatty acids and polyunsaturated fatty acids (PUFA) compared to beef, which could be a reason for why some panelists noted a gamey flavor at week 0. Panelists noted that bars and bites had gamey, musty, and rancid flavors, which agrees with findings from Larick *et al.* (1989) and Koch *et al.* (1989).

Table 2-3. Least squares means for sensory and instrumental attributes for ready-to-eat bison bars¹ evaluated over 18 weeks of accelerated storage at 40°C.

Attributes	Week					
	0	3	5	12	18	SEM ²
External Color ³	5.3 ^d	5.9 ^c	5.7 ^{cd}	6.7 ^b	7.6 ^a	0.18
Bite ⁴	4.1	3.6	3.5	3.2	4.8	0.49
Tenderness ⁵	3.3	2.8	2.9	3.1	4.2	0.37
Sweetness ⁶	3.1 ^a	3.1 ^a	2.8 ^a	2.7 ^a	2.1 ^b	0.20
Saltiness ⁷	2.6 ^{bc}	3.0 ^a	2.9 ^a	2.4 ^{ab}	2.4 ^{cd}	0.11
Fruit Flavor ⁸	3.1 ^a	2.9 ^a	2.9 ^a	2.8 ^{ab}	2.1 ^b	0.18
Sour ⁹	2.2	2.4	2.7	2.4	2.8	0.18
Off-Flavor ¹⁰	1.7 ^b	2.2 ^b	2.5 ^b	3.1 ^b	4.2 ^a	0.42
Warner Bratzler Shear Force ¹¹	8.83	9.26	5.57	5.66	10.9	2.74

^{a-d} Least squares means with different letters within rows are significantly different ($P<0.05$).

¹ n=2.

²±Standard error of the mean.

³External color 1= extremely light, 8 = extremely dark.

⁴Bite 1= extremely soft, 8 = extremely hard.

⁵Tenderness 1= extremely Tender, 8= extremely tough.

⁶Sweetness 1= no sweetness, 8= extremely sweet.

⁷Saltiness 1 = not salty, 8= extremely salty.

⁸Fruit flavor 1 = no fruit flavor, 8=extreme fruit flavor.

⁹Sourness 1= no sourness, 8=extremely sour.

¹⁰ Off-Flavor 1= no off flavor, 8=extreme off flavor.

¹¹Warner Bratzler shear force = kilograms of force.

Sensory Attributes for Bites

Probability values for the week of storage for sensory attributes for bites during accelerated storage are summarized in Appendix C-4.

External Color

Sensory panelists found that bites became visually lighter ($P<0.05$) from week 0 to week 3. Bite color remained similar ($P>0.05$) from weeks 3 through 5. From weeks 5 to 12, panelists found that bites darkened and remained darker ($P<0.05$) until week 18 (Table 2-4).

Texture

Sensory panelists found that bites decreased ($P<0.05$) in bite force from week 0 to week 3, and then bites remained softer ($P>0.05$) from week 3 to week 18 (Table 2-4). Bites became more tender ($P<0.05$) from week 0 through week 3, and they remained tender ($P>0.05$) from week 3 to week 18 (Table 2-4). Bites presented with no differences ($P>0.05$) in WBSF from week 0 through week 18 (Table 2-4).

Flavor Intensity

Sensory panelists found that bite sweetness remained similar ($P>0.05$) from week 0 through 18, although sweetness tended to decrease during storage (Table 2-4). Panelists found that bite, saltiness fruit flavor, and sourness remained similar ($P>0.05$) from week 0 through 18 (Table 2-4). Panelists were unable to perceive any changes in sourness while pH changed over time (Table 2-2). Off flavor for bites tended to increase but was not different ($P>0.05$) from week 0 to week 5 and then increased to 3.6 by week 18 (Table 2-4).

Table 2-4. Least squares means for sensory attributes for ready-to-eat bison bites¹ evaluated over 18 weeks of accelerated storage at 40°C.

Attributes	Week					SEM ²
	0	3	5	12	18	
External Color ³	5.5 ^b	4.5 ^c	4.4 ^c	6.1 ^a	6.6 ^a	0.21
Bite ⁴	3.7 ^a	2.7 ^{bc}	2.2 ^c	2.9 ^b	3.0 ^b	0.18
Tenderness ⁵	3.2 ^a	2.3 ^{bc}	1.9 ^c	2.6 ^b	2.5 ^b	0.15
Sweetness ⁶	3.5	2.9	3.0	2.8	2.6	0.20
Saltiness ⁷	2.5	2.5	2.7	2.7	2.4	0.14
Fruit Flavor ⁸	3.4	2.9	2.9	2.9	2.6	0.21
Sour ⁹	2.2	2.6	2.3	2.4	2.2	0.14
Off Flavor ¹⁰	1.5 ^c	2.1 ^{bc}	2.1 ^{bc}	2.3 ^b	3.6 ^a	0.22
Warner Bratzler Shear Force ¹¹	5.44	5.18	3.11	5.93	5.25	1.59

^{a-c} Least squares means with different letters within rows are significantly different ($P<0.05$).

¹ n=2.

² Standard error of the mean.

³ External color 1= extremely light, 8 = extremely dark.

⁴ Bite 1= extremely soft, 8 = extremely hard.

⁵ Tenderness 1= extremely Tender, 8= extremely tough.

⁶ Sweetness 1= no sweetness, 8= extremely sweet.

⁷ Saltiness 1 = not salty, 8= extremely salty.

⁸ Fruit flavor 1 = no fruit flavor, 8=extreme fruit flavor.

⁹ Sourness 1= no sourness, 8=extremely sour.

¹⁰ Off flavor 1= no off flavor, 8=extreme off flavor.

¹¹ Warner Bratzler shear force = kilograms of force.

Instrumental Color for Bars

Probability values for the week of storage for instrumental color for bars during accelerated storage are summarized in Appendix C-5. Bar lightness remained similar ($P>0.05$) from week 0 through week 18 (Table 2-5). Bars became less red ($P<0.05$) from 20.6 at week 0 to 4.77 by week 18 (Table 2-5). Bars became less yellow ($P<0.05$) from 25.4 at week 0 to 4.42 by week 18 (Table 2-5). The a*/b* ratio for bars remained similar ($P>0.05$) with a ratio of 0.82 at week 0 to 0.84 at week 3 (Table 2-5). This ratio decreased ($P<0.05$) from 0.84 at week 3 to 0.81 at week 5, and then increased ($P<0.05$) from 0.81 at week 5 to 1.44 at week 12 (Table 2-5).

The a^*/b^* ratio remained similar ($P>0.05$) from week 12 to 1.08 by week 18. Saturation index ($((C=a^{*2}+b^{*2}) 0.5)$) indicates intensity of hue color in products (AMSA 2012). Bars became less vivid ($P<0.05$) with a value of 6.76 at week 0 to 3.01 by week 18 (Table 2-5). Hue angle for bars remained similar ($P>0.05$) with a value of 50.9 at week 0 and 51.2 at week 5. Bars became more discolored ($P<0.05$) from week 5 to week 12 when the hue angle was 36.8, and the hue angle remained similar from week 12 through week 18 with a final hue angle of 43.8 (Table 2-5). Although sensory panelists perceived the bars to become darker by week 18 of storage, this was not detected by instrumental determination of L^* . It is possible that there were mechanical errors with the instrumental observations due to technical difficulties with the MiniScan.

Table 2-5. Least squares means for instrumental color for ready-to-eat bison bars¹ evaluated over 18 weeks of accelerated storage at 40°C.

Attributes	Week						SEM ²
	0	3	5	12	14	18	
L^* ³	22.4	18.4	16.1	15.8	13.7	22.6	2.98
a^* ⁴	20.6 ^a	21.9 ^a	18.3 ^a	13.3 ^b	10.5 ^b	4.77 ^c	1.71
b^* ⁵	25.4 ^{ab}	26.2 ^a	22.7 ^b	9.53 ^c	7.77 ^c	4.42 ^d	0.97
a^*/b^* Ratio ⁶	0.82 ^b	0.84 ^b	0.81 ^c	1.44 ^a	1.38 ^a	1.08 ^{ab}	0.13
Saturation Index ⁷	6.76 ^a	6.93 ^a	6.39 ^a	4.69 ^b	4.17 ^b	3.01 ^c	0.28
Hue Angle ⁸	50.9 ^a	50.1 ^a	51.2 ^a	36.8 ^b	38.1 ^b	42.8 ^b	2.34

^{a-d} Least squares means with different letters within rows are significantly different ($P<0.05$).

¹ n=2.

² Standard error of the mean.

³ L^* Lightness (white 100 = black 0).

⁴ a^* Redness/green ness (red 60 = green -60).

⁵ b^* yellowness/blueness (yellow 60= blue -60).

⁶ a^*/b^* ratio.

⁷ Saturation index ($((C=a^{*2}+b^{*2}) 0.5)$).

⁸ Hue angle ($h=\arctangent(b^*/a^*)$).

Instrumental Color for Bites

Probability values for the week of storage for instrumental color for bites during accelerated storage are summarized in Appendix C-6. Initial L^* for bites remained similar ($P>0.05$) from week 0 through week 18 (Table 2-6). Bite redness remained similar ($P>0.05$) from week 0 through week 12. Bites became less red ($P<0.05$) by week 14, and then remained

similar ($P>0.05$) through week 18 (Table 2-6). Bite yellowness increased ($P<0.05$) from week 0 to week 3, and then slightly decreased ($P>0.05$) at week 12; however, week 12 was similar ($P>0.05$) to week 0 (Table 2-6). Bites became less yellow ($P<0.05$) from week 12 to week 14, and then remained similar ($P>0.05$) through week 18; however, week 0 was similar ($P>0.05$) to weeks 14 and 18 (Table 2-6). Bite a^*/b^* ratio decreased ($P<0.05$) from week 0 until week 3 (Table 2-6). The a^*/b^* ratio was similar ($P>0.05$) for weeks 0, 12, 14, and 18 (Table 2-6). Bite saturation index remained similar ($P>0.05$) from week 0 to week 3, and then saturation index remained similar ($P>0.05$) from week 3 through week 5. Bites at week 5 were significantly more vivid ($P<0.05$) than at week 0 (Table 2-6). Bites became less vivid ($P<0.05$) from week 5 through week 14, and then remained similar ($P>0.05$) from week 14 to week 18 (Table 2-6). Bite hue angle increased ($P<0.05$) from week 0 until week 3. Bites had a similar ($P>0.05$) hue angle for weeks 0, 12, 14, and 18 indicating a similar amount of discoloration (Table 2-6). Although sensory panelists perceived the bites to become darker by week 18 of storage, this was not detected by instrumental determination of L^* . It is possible that there were mechanical errors with the instrumental observations due to technical difficulties with the MiniScan.

Table 2-6. Least squares means for instrumental color for ready-to-eat bison bites¹ evaluated over 18 weeks of accelerated storage at 40°C.

Attributes	Week						SEM ²
	0	3	5	12	14	18	
L^* ³	27.9	23.9	26.6	21.7	27.2	25.9	1.55
a^* ⁴	15.5 ^{ab}	17.9 ^{ab}	19.1 ^a	14.4 ^b	10.1 ^c	8.55 ^c	1.42
b^* ⁵	16.1 ^{bc}	23.9 ^a	25.1 ^a	18.4 ^{ab}	10.5 ^c	8.82 ^c	2.46
a^*/b^* Ratio ⁶	1.01 ^a	0.75 ^b	0.77 ^b	0.86 ^{ab}	0.98 ^a	0.98 ^a	0.06
Saturation Index ⁷	5.57 ^b	6.44 ^{ab}	6.64 ^a	5.62 ^b	4.51 ^c	4.15 ^c	0.33
Hue Angle ⁸	45.3 ^b	53.2 ^a	52.7 ^a	49.9 ^{ab}	45.8 ^b	45.6 ^b	1.90

^{a-c} Least squares means with different letters within rows are significantly different ($P<0.05$).

¹n=2.

²Standard error of the mean.

³ L^* Lightness (white 100 = black 0).

⁴ a^* Redness/green ness (red 60 = green -60).

⁵ b^* Yellowness/blueness (yellow 60= blue -60).

⁶ a^*/b^* ratio.

⁷Saturation index ((C= $a^*^2+b^*^2$) 0.5).

⁸Hue angle ($h = \arctangent(b^*/a^*)$).

Conclusions

The recommended shelf life for bars would be equivalent to 5 months at ambient temperature based on 5 weeks at accelerated storage at 40°C. Overall, attributes for bars were consistent from week 0 to week 5 for: a_w , pH, sensory bite, tenderness, sweetness, saltiness, fruit flavor, sourness, and external instrumental color. After 5 weeks of accelerated storage bars darkened, lost moisture, and became less flavorful over time. The recommended shelf life for bites would be equivalent to 5 months at ambient temperature based on 5 weeks at accelerated storage at 40°C. Overall, attributes for bites were consistent from week 0 to week 5 for: a_w , sweetness, saltiness, fruit flavor, sourness, off flavor, and external instrumental color. After 5 weeks of accelerated storage bites darkened and lost moisture.

Bibliography

- AMSA. (2012). Guidelines for Measuring Meat Color. American Meat Science Association, Champaign, IL. <http://www.meatscience.org>.
- Argyri, A. A., Panagou, E. Z., & Nychas, G. J. E. (2012). In Kerry J. P. (Ed.), *Advances in vacuum and modified atmosphere packaging of poultry products*.
- Barsh, R. L., & Marlor, C. (2003). Driving bison and blackfoot science. *Human Ecology*, 31(4), 571-593.
- Chawla, S. P., & Chander, R. (2004). Microbiological safety of shelf-stable meat products prepared by employing hurdle technology. *Food Control*, 15(7), 559-563.
- Coma, V. (2006). Advanced technologies for meat processing In M. L. L. Nollet, & F. Toldra (Eds.), *Perspectives for the active packaging of meat products*. pp.449-468. Boca Raton: CRC Press.
- Corradini, M. G., & Peleg, M. (2007). Shelf-life estimation from accelerated storage data. *Trends in Food Science & Technology*, 18(1), 37-47.
- Cronin, M. A., MacNeil, M. D., Vu, N., Leesburg, V., Blackburn, H. D., & Derr, J. N. (2013). Genetic variation and differentiation of bison (bison bison) subspecies and cattle (bos taurus) breeds and subspecies. *Journal of Heredity*, 104(4), 500-509.
- Esse, R., & Saari, A. (2004). In Steele R. (Ed.), *Shelf-life and moisture management*. Philadelphia, PA: Woodhead Publishing.
- Freese, C. H., Aune, K. E., Boyd, D. P., Derr, J. N., Forrest, S. C., Gates, C. C., Gogan, P. J., Grassel, S. M., Halbert, N. D., Kunkel, K., Redford, K. H. (2007). Second chance for the plains bison. *Biological Conservation*, 136(2), 175-184.
- Friedman, M. (1996). Food browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry*, 44(3), 631-653.
- Harper, N. M., Getty, K. J. K., & Boyle, E. A. E. (2010). Evaluation of sample preparation methods for water activity determination in jerky and kippered beef: A research note. *Meat Science*, 86(2), 527-528.
- Joseph, P., Suman, S. P., Li, S., Beach, C. M., Steinke, L., & Fontaine, M. (2010). Characterization of bison (bison bison) myoglobin. *Meat Science*, 84(1), 71-78.

- Karel, M., & Labuza, T. P. (1968). Nonenzymatic browning in model systems containing sucrose. *Journal of Agricultural and Food Chemistry*, 16(5), 717-719.
- Kesava Rao, V., Kowale, B. N., Murthy, T. R., & Sharma, N. (1992). Effect of processing and storage on neutral lipids of buffalo meat. *Meat Science*, 31(1), 25-34.
- Kilcast, D., & Subramaniam, P. (2000). *Stability and shelf-life of food*. Philadelphia, PA: Woodhead Publishing.
- Koch, R. M., Jung, H. G., Crouse, J. D., Varel, V. H., & Cundiff, L. V. (1995). Growth, digestive capability, carcass and meat characteristics of bison-bison, bos-taurus, and bos X bison. *Journal of Animal Science*, 73(5), 1271-1281.
- Labuza, T. P., Acott, K. M., & Sloan, A. E. (1974). Intermediate moisture foods; chemical and nutrient stability. *IV International Congress of Food Science and Technology*, 7a, 1-3.
- Labuza, T. P. (1982a). Moisture gain and loss in packaged foods. *Food Technology*, 36(4), 92-96.
- Labuza, T. P. (1982b). *Shelf-life dating of foods*. Westport, CT: Food & Nutrition Press.
- Labuza, T. P., & Schmidl, M. K. (1985). Accelerated shelf-life testing of foods. *Food Technology*, 39(9), 57-64.
- Labuza, T. P., & Szybist, L. M. (2001). *Open dating of foods*. Trumbull, CT: Food & Nutrition Press.
- Larick, D. K., Turner, B. E., Koch, R. M., & Crouse, J. D. (1989). Influence of phospholipid content and fatty-acid composition of individual phospholipids in muscle from bison, hereford and brahman steers on flavor. *Journal of Food Science*, 54(3), 521-526.
- Leonard, S. M., & Larick, D. K. (1990). Effect of vacuum-packaged frozen storage, after cooking, on the phospholipids of hereford and bison. *Meat Science*, 28(4), 299-311.
- Malik, A. H., & Sharma, B. D. (2010). Comparison of hurdle treatments for buffalo meat. *International Journal of Food Science and Technology*, 45(8), 1552-1563.
- Malik, A. H., & Sharma, B. D. (2014). Shelf life study of hurdle treated ready-to-eat spiced buffalo meat product stored at 30 +/- 3 degrees C for 7 weeks under vacuum and aerobic packaging. *Journal of Food Science and Technology-Mysore*, 51(5), 832-844.

- Marchello, M. J., Slanger, W. D., Hadley, M., Milne, D. B., & Driskell, J. A. (1998). Nutrient composition of bison fed concentrate diets. *Journal of Food Composition and Analysis*, 11(3), 231-239.
- Marchello, M. J., Slanger, W. D., Milne, D. B., Fischer, A. G., & Berg, P. T. (1989). Nutrient composition of raw and cooked bison-bison. *Journal of Food Composition and Analysis*, 2(2), 177-185.
- Marchello, M. J., & Driskell, J. A. (2001). Nutrient composition of grass- and grain-finished bison. *Great Plains Research*, 11(1), 65-82.
- Mizrahi, S. (2011). In Kilcast D. S.,P. (Ed.), *Accelerated shelf life testing of foods*. Philadelphia, PA: Woodhead Publishing.
- Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry*, 62(4), 415-424.
- National Bison Association (2014). Westminster, CO. <http://www.bisoncentral.com>.
- Obanu, Z. A., Ledward, D. A., & Lawrie, R. A. (1975a). The protein of intermediate moisture meat stored at tropical temperature. I. changes in solubility and electrophoretic pattern. *Journal of Food Technology*, 10(6), 657-666.
- Obanu, Z. A., Ledward, D. A., & Lawrie, R. A. (1975b). The protein of intermediate moisture meat stored at tropical temperature. II. effect of protein changes on some aspects of meat quality. *Journal of Food Technology*, 10(6), 667-674.
- Okonkwo, T. M., Obanu, Z. A., & Ledward, D. A. (1992a). Characteristics of some intermediate moisture smoked meats. *Meat Science*, 31(2), 135-145.
- Okonkwo, T. M., Obanu, Z. A., & Ledward, D. A. (1992b). The stability of some intermediate moisture smoked meats during storage at 30-degrees-C and 38-degrees-C. *Meat Science*, 31(3), 245-255.
- O'Sullivan, M. G., & Kerry, J. P. (2012). In Kerry J. P. (Ed.), *Sensory and quality properties of packaged fresh and processed meats* (86-104). Philadelphia, PA: Woodhead Publishing.
- Roudaut, G., & Debeaufort, F. (2011). In Kilcast D. S.,P. (Ed.), *Moisture loss, gain and migration in foods* (63-100). Philadelphia, PA: Woodhead Publishing.

- Rule, D. C., Broughton, K. S., Shellito, S. M., & Maiorano, G. (2002). Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. *Journal of Animal Science*, 80(5), 1202-1211.
- Sahoo, J., Kumar, S., & Yadav, P. L. (1994). Developments in technology of intermediate moisture foods with special reference to indian meat industry. *Indian Food Industry*, 13(3), 35-45.
- Sebranek, J., & Houser, T. (2006). Advanced technologies for meat processing. In Nollet M. L. Leo, & Toldra Fidel (Eds.), *Modified atmosphere packaging*. (pp. 419-472). Boca Raton: CRC Press.
- Sell, R. S., Bangsund, D. A., & Leistritz, F. L. (2001). Contribution of the bison industry to north dakota's economy. *American Journal of Alternative Agriculture*, 16(3), 106-113.
- Thomas, P. L. (1975). Dried meat products. *CSIRO Food Research Quarterly*, 35(4), 73-78.
- Tyrer, H., Ainsworth, P., Ibanoglu, S., & Bozkurt, H. (2004). Modeling the growth of *pseudomonas fluorescens* and *candida sake* in ready-to-eat meals. *Journal of Food Engineering*, 65(1), 137-143.
- Waletzko, P., & Labuza, T. P. (1976). Accelerated shelf-life testing of an intermediate moisture food in air and in an oxygen-free atmosphere. *Journal of Food Science*, 41(6), 1338-1344.
- Webster, C. E. M., Ledward, D. A., & Lawrie, R. A. (1982). Effect of oxygen and storage-temperature on intermediate moisture meat-products. *Meat Science*, 6(2), 111-121.
- Zhang, Y. G., Ritter, W. J., Barker, C. C., Traci, P. A., & Ho, C. T. (1994). Volatile formation by lipid-mediated maillard reaction in model systems. *Lipids in Food Flavors*, (Vol. 558) (pp. 49-60).

Appendix A - Sensory Evaluation Form

A large grid of 10 columns and 6 rows of gray bars, likely a sensory evaluation form for tasting or smelling. The grid is composed of vertical and horizontal bars of varying widths and shades of gray, creating a pattern of small rectangles. The first column contains a single row of bars. The second through tenth columns each contain two rows of bars. The top row of bars in the second through tenth columns is significantly longer than the bottom row.

Appendix B - Statistical Code for Bars and Bites

Sensory Analysis

```
proc mixed data=work.b;  
  class week;  
  model _color=week;  
  lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
  class week;  
  model bite=week;  
  lsmeans week /pdiff;  
run;
```

```
proc mixed data=work.b;  
  class week;  
  model tenderness=week;  
  lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
  class week;  
  model sweetness=week;  
  lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
  class week;  
  model saltiness=week;
```

```
lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
class week;  
model fruit=week;  
lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
class week;  
model off=week;  
lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
class week;  
model sour=week;  
lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
class week;  
model shear=week;  
lsmeans week/pdiff;  
run;
```

Physiochemical Analysis

```
proc mixed data=work.b;
```

```
class week;  
model pH=week;  
lsmeans week/pdiff;  
run;  
  
proc mixed data=work.b;  
class week;  
model moisture=week;  
lsmeans week/pdiff;  
run;  
  
proc mixed data=work.b;  
class week;  
model protein=week;  
lsmeans week/pdiff;  
run;  
  
proc mixed data=work.b;  
class week;  
model aw=week;  
lsmeans week/pdiff;  
run;  
  
proc mixed data=work.b;  
class week;  
model apple=week;  
lsmeans week/pdiff;  
run;  
  
proc mixed data=work.b;
```

```
class week;  
model cran=week;  
lsmeans week/pdiff;  
run;  
  
proc mixed data=work.b;  
class week;  
model MPR=week;  
lsmeans week/pdiff;  
run;
```

Instrumental Color Analysis

```
proc mixed data=work.b;  
class week;  
model L=week;  
lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
class week;  
model A=week;  
lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;  
class week;  
model B=week;  
lsmeans week/pdiff;  
run;
```

```
proc mixed data=work.b;
class week;
model a/b=week;
lsmeans week/pdiff;
run;
```

```
proc mixed data=work.b;
class week;
model SI=week;
lsmeans week/pdiff;
run;
```

```
proc mixed data=work.b;
class week;
model hue=week;
lsmeans week/pdiff;
run;
```

Appendix C - Probability Tables

Table C-1. Probability values for week of storage for physicochemical properties for ready to eat bison bars during accelerated storage at 40°C.

Attributes	P-value
Water Activity	0.0862
Percent Moisture	0.0030
Percent Protein	0.4453
MPR	0.0029
pH	0.0092

Table C-2. Probability values for week of storage for physicochemical properties for ready to eat bison bites during accelerated storage at 40°C.

Attributes	P-value
Water Activity	0.0011
Percent Moisture	0.0014
Percent Protein	0.0012
MPR	0.0002
pH	0.0017

Table C-3. Probability values for week of storage for sensory and instrumental attributes for ready to eat bison bars during accelerated storage at 40°C.

Attributes	P-value
External Color	<0.0001
Bite	0.2113
Tenderness	0.1114
Sweetness	0.0092
Saltiness	0.0082
Fruit Flavor	0.0144
Sour	0.2048
Off-Flavor	0.0070
Warner Bratzler Shear Force	0.5239

Table C-4. Probability values for week of storage for sensory and instrumental attributes for ready to eat bison bites during accelerated storage at 40°C.

Attributes	P-value
External Color	<0.0001
Bite	0.0006
Tenderness	0.0004
Sweetness	0.0546
Saltiness	0.5393
Fruit Flavor	0.1083
Sour	0.1693
Off-Flavor	0.0001
Warner Bratzler Shear Force	0.3425

Table C-5. Probability values for week of storage for instrumental color for ready to eat bison bars during 18 weeks of accelerated storage at 40°C.

Attributes	P-value
L*	0.2373
a*	<0.0001
b*	<0.0001
a*/b* Ratio	0.0070
Saturation Index	<0.0001
Hue Angle	0.0004

Table C-6. Probability values for week of storage for instrumental color for ready to eat bison bites during 18 weeks of accelerated storage at 40°C.

Attributes	P-value
L*	0.0945
a*	0.0002
b*	0.0006
a*/b* Ratio	0.0319
Saturation Index	0.0002
Hue Angle	0.0177